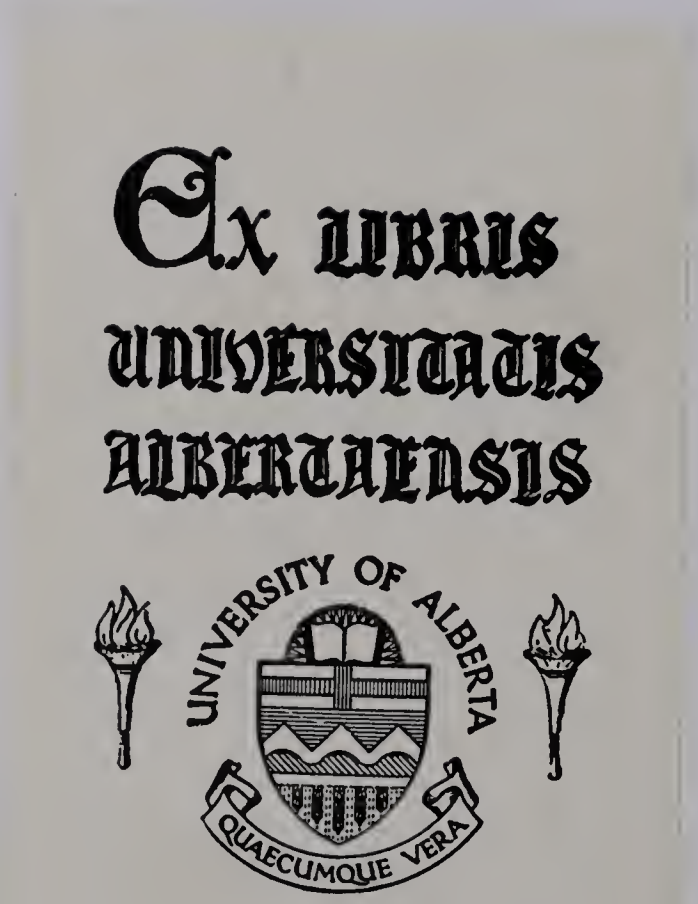


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# INFLUENCE OF (2-CHLOROETHYL) TRIMETHYLAMMONIUM CHLORIDE ON SOME ASPECTS OF GROWTH AND DEVELOPMENT OF *Lycopersicon esculentum* L. and *Cucumis sativus* L.

FACULTY OF GRADUATE STUDIES
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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled: "Influence of (2-Chloroethyl) Trimethylammonium Chloride on Some Aspects of Growth and Development of *Lycopersicon esculentum* L. and *Cucumis sativus* L." submitted by Joseph Michael Molnar, in partial fulfulment of the requirements for the degree of Master of Science.

## ABSTRACT

This thesis reports the influence of (2-chloroethyl) trimethylammonium chloride (CCC) on the early yields of Lycopersicon esculentum L., on the sex expression of Cucumis sativus L., and on some morphological effects and the chlorophyll and phosphorus content of tomatoe leaves.

It was found in the field experiments that CCC applied to tomato plants (Early Alberta), as a soil drench 10 days before, and again at field setting time, increased the early tomato yields 50% compared to control.

In the greenhouse, similar results were observed with the Tuck Queen variety when CCC was applied as a soil drench. Foliar applications increased the early yields four times compared to control in the first two weeks of harvest.

CCC applications increased the flower number in the first cluster in both varieties, and in most cases the first flower cluster appeared at a lower node number.

The number of days from germination to the first inflorescence was reduced by 5 days as a result of CCC foliar treatments. The treatments also increased the production of parthenocarpic fruit in the greenhouse experiments.

The height of the plants was reduced in both varieties and root development was reduced when the plants were treated with 500 ppm CCC as a soil drench. In both cases, the magnitude

of the effect increased as the CCC concentrations were increased.

The chlorophyll content increased as a result of CCC applications and total phosphorus content of tomato leaves appeared to be decreased due to CCC treatments.

Field experiments involving the application of CCC to three varieties of cucumber, did not produce any observable difference between treated and untreated plants. Greenhouse experiments, however, with two varieties (Marketer and Hybrid Burpee) indicated some measurable differences.

The number of pistillate flowers did not increase, but the staminate flower formation was strongly inhibited in both varieties. The yield was not affected when CCC was applied at low concentrations, but high concentrations reduced the total yields 50% compared to the control.

The height of the CCC treated plants was significantly reduced at all concentrations used. The measurable differences in height were much greater in winter than in summer.

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# I. INTRODUCTION

Any chemical treatment that is capable of inducing earlier yields or increasing the total yields of tomatoes and cucumbers, could be of considerable importance to the vegetable industry, particularly in those regions where a comparatively short growing season is an important limiting factor in production.

Recent findings have shown that crop yields and the quality of plants may be affected directly by the use of appropriate growth regulators.

One such growth regulator (2-chloroethyl) trimethylammonium chloride (CCC) has been shown to modify the growth and development of tomato plants (50). For example, flowering was three to ten days earlier and the stem height to the first flower cluster was greatly reduced. The plants were stockier and darker green in color. The flower number on the first inflorescence was increased (50). CCC has also been reported to increase the number of pistillate flowers of cucumber plants (37).

This thesis deals with some effects of (2-chloroethyl) trimethylammonium chloride (CCC) on tomatoes and cucumbers.

The two major objectives of the investigation were to determine, under Alberta growing conditions, the effect of CCC on:

1) The growth and development of tomatoes.

2) The growth and sex expression of cucumbers.

## II. REVIEW OF LITERATURE

### A. HISTORY OF CCC.

In 1960 a new group of quaternary ammonium compounds was reported by Tolbert (43). The most active compound was (2-chloroethyl) trimethylammonium chloride (CCC). This chemical retarded the growth of a larger number of species than any of the earlier compounds. One chemical related to this group had been reported previously by Schweiner and Reed in 1908 (31). They noted that plants were injured when grown in solutions containing 25 ppm of (ethyl) trimethylammonium bromide, and were killed by 250 ppm of this compound. However, no other experiments were reported with plants until 1960 (43). (2-Chloroethyl) trimethylammonium chloride is an analog of choline, and in that the hydroxy group was replaced with a choline substitute. Its name was chlorocholine chloride, abbreviated to CCC, and it is now marketed commercially under the name of Cycocel.

### B. CHEMICAL AND PHYSICAL PROPERTIES OF CCC.

CCC is a stable white solid, soluble in water and lower alcohols. Its empirical formula is $C_5 H_{13} Cl_2 N$ (44), and the structural formula is as follows:

$$\begin{array}{cccccc} & & H & H & CH_{3+} & \\ Cl & C & C & N & CH_{3+} & .Cl^- \\ & & H & H & CH_3 & \end{array}$$

The molecular weight is 158.1 and it has a melting point of 245°C

(decomposition) (37). CCC is formulated as a 50% (W/V) aqueous solution which is chemicallystable and retains its biological effectiveness. It does not present health hazards in research work and is phytotoxic only if applied in a very high dosage and its mammalian toxicity is also relatively low. It persists in soil for one crop only or less. Steam sterilization eliminates the chemical, thus treated soils can be safely re-used. Mitchell suggested that micro-organisms also caused the destruction of CCC in root medium (25).

C. METHOD OF APPLICATION OF CCC.

The chemical is most effective when applied to actively growing plant parts. To date the most efficient utilization has been through the roots, applied as a soil drench or by direct incorporation in the soil. Seed treatment, in certain cases, has also been reported as successful (41). Tiessen (40) has reported the application of foliar spray on peppers and tomatoes, without reducing the height of the plants. Cathey and Stuart, working with poinsettias, obtained results with the spray application similar to those obtained by soil drenches.

Tolbert (44) treated wheat plants with CCC, and noted that when the chemical was applied as a spray, it was less affective than the drench application at the same molarity. He suggests that the reason for this is the fact that large volumes of dilute solutions could be applied to the soil as a more continuous source. When he placed seeds on filter paper overnight, moistened with an

aqueous solution of CCC, and planted them the following morning in untreated soil, the appearance of these plants was similar to that obtained from the treatment of seedlings.

One application, or at the most two, made within the first weeks of growth were usually sufficient to affect the plants until maturity (37).

## D. PHYSIOLOGICAL EFFECTS OF CCC ON PLANTS.

### 1. Flower initiation and flower quality.

In certain plants the time of flower initiation is greatly accelerated by growth retardants. Stuart (35) first demonstrated that the application of the growth retardant CCC, caused suppression of vegetative growth and prompt initiation of flower buds in rhododendrons. These results were confirmed by Cathey and Taylor (6) who observed that CCC treated plants produced abnormal inflorescences.

Lindstrom and Tolbert (22) found similar abnormal inflorescence results in studying flower formation of poinsettias and chrysanthemums. They also observed less wilting during growth and improved keeping quality of the flowers after flowering (22).

CCC treatments increased the keeping quality of cut flowers. Halevy and Wittwer (16) reported that overnight immersion of the stem bases in CCC solutions of 10, 50 and 100 ppm levels, significantly increased the life of carnations and snapdragons over the control. The results demonstrated that the ageing of cut flowers may also be induced by appropriate treatments with CCC. The

effects of overnight treatment persisted throughout the whole life of the flower.

Acceleration of flowering was observed in other herbaceous plants (1,40,41,50). Tiessen observed that tomato seedlings treated with CCC in the cotyledon stage (39), flowered earlier, and produced a larger number of flowers in the first inflorescence than untreated plants. Treatments also promoted flowering at a lower node than that which occurred on untreated plants.

Flower initiation of Bryophyllum doigremontianum (54) was fully suppressed by the application of CCC and related compounds.

Zeevaart (53) in 1964 reported the effects of CCC on a short day plant - Pharbitis nil, strain violet. Flowering in this short day plant is suppressed by soil drench of CCC. The inhibition can be overcome by the application of gibberellin (GA). When the plant is grown under long day conditions, CCC promotes flowering. Available evidence suggests that CCC inhibits flower formation in Pharbitis nil by inhibiting cell division in the plumule during the period when the floral stimulus is present.

According to Cathey (4), growth retardants apparently promote flowering by modifying activity in the cambium. This results in abnormal types of cells in the xylem and the disappearance of sclerenchymatus cells adjacent to the cortex. The restriction of growth presumably alters the metabolism and creates conditions conducive to flower initiation.

2. Effects of CCC on sex expression.

Wittwer (37) treated Cucumis sativus var. Marketer with CCC.

He applied 100 mls. of solution per 4" pot at $10^{-3}$ - $10^{-5}$ M concentrations, and reported an increase in the number of female flowers. Cathey and Stuart (5,37) mixed 4, 10 and 20 mg. of CCC per cubic foot of soil to test the reaction of the Marketer cucumber and observed only retardation of plant growth, with no affect on the sex ratio.

Later, several modifications of CCC were used to study the effects on growth and flowering of cucumber plants. Ota (4) in Japan, used (2-bromoethyl) trimethylammonium bromide (BCB) and Mitchell (25) used allyltrimethylammonium bromide (ANAB) to retard stem elongation, promote lateral shoot development, and alter the position and number of staminate and pistillate flowers. These compounds inhibited staminate flower production, greatly increased pistillate flower formation and occasionally promoted the formation of hermaphroditic flowers. The node locations of the first pistillate flower and the first continuous pistillate flower were lowered as compared with the position on untreated plants. As a result of the chemical modifications of the plant, the position of the first tendril and the first pistillate flower were negatively correlated.

Mitchell (25) suggested that growth retardants were not causal agents of flower sex expression, but that the influence may be a result of regulated vegetative growth.

3. Chemical composition.

Treatment of barley seedlings (4) with CCC altered the trans-

location of phosphorus. Tolbert reported that 30% of total soluble phosphorus in plant roots and leaves is present as phosphorylcholine. The application of CCC to the nutrient solution 24 to 72 hours before the addition of $P^{32}$ reduced its specific activity in leaves 75 to 95% in comparison with plants growing in water, or comparable molarities of sodium chloride or manitol. The total $P^{32}$ in the CCC treated seedlings was three to four times as large, primarily because of the great increase of $P^{32}$ in the roots.

In tobacco plants, Humphries (19) reported an increased chlorophyll content, both per leaf and per unit area, and increased dry weight per unit area. Norris (28) also reported increased chlorophyll content in grass leaves due to CCC treatment. Corn seedlings grown in a CCC solution had a higher chlorophyll content than seedlings grown in a solution without CCC. This effect appeared to be due to an increased rate of proto-chlorophyll synthesis.

Miller (25) observed that CCC treatment significantly increased the chlorophyll content of barley.

4. Increased resistance to chemical and physical changes and diseases.

Halevy (14) reported in 1963 increased tolerance of bean plants to soil drought, by means of growth retarding substances. He treated wax beans with CCC and phosfon. The seedlings were grown in a container and the chemical was applied by drench application, 250 ml. containing 50 mg. phosfon or 500 mg. CCC. Control leaves began to fall off after 9 days, whereas CCC treated plants remained turgid for 30 days. Leaves of phosfon treated plants started falling

42 days after the last watering. After 42 days the soil moisture was 4.36% (phosfon), 3.98% (CCC) and 3.62% in control.

Wunsche and Wittwer (52) also observed differences with water requirements. Potometer experiments with bean plants treated with CCC revealed that water uptake per unit leaf area was reduced by one half. Similar results were obtained with wheat and barley. The differences in water requirements became apparent within 1 day after treatment, and reached the maximum within 3 to 5 days.

Norris (28) also noted that CCC tended to increase the ability of grasses to recover from clipping, and also possibly their resistance to drought. Corn seedlings, grown in high concentrations of CCC, had a lower water content than those grown in water only.

Gohlhe and Tolbert (11) grew young barley seedlings in nutrient solution containing CCC. Water uptake by the seedlings was measured with a potometer and found to be inhibited 60 to 80% by $10^{-4}$ to $10^{-3}$ M CCC. Water translocation, as measured by the quantity of xylem exudate, was similarly inhibited.

Young soy bean and wheat plants treated with CCC showed a marked increase in resistance to high salt content and the pH change in the soil. Miyamoto (27) soaked wheat seeds in 0.5% of CCC for 14 hours and planted them in sandy soil (pH 6.45). After 9 days he added sodium hydroxide to increase the pH to 11.98, or sulphuric acid to decrease it to 3.24. Low and high pH caused curling of the leaf blades, bleaching of leaf color and wilting of untreated plants. The CCC treated plants showed

increased resistance to high and low pH values.

Van Emden (46) reported that CCC treated brussel sprouts were not as heavily infested with cabbage aphids as the control plants. According to Sinha and Wood (32), CCC increased the resistance of susceptible tomato varieties to the fungus Verticillium which causes wilting of the tomato plants. In this case it was shown that the resistance was due to changes in the host, as CCC treated cultures of the fungus grew better than untreated ones. In both these reports the authors suggested that CCC might have altered the host plant physiology in such a way that the parasite could not successfully compete any longer. Rawlins (Cathey's review 4), demonstrated that treatments of tobacco discs with CCC lowered the multiplication of the tobacco mosaic virus.

5. Persistence in plants.

The persistence of CCC in plants was shown by Zeevaart in 1965 (55) by the retardation of Japanese Morning Glory, Pharbitis nil, grown from seed harvested from CCC treated plants. The seedlings obtained from these seeds were strongly dwarfed. This could have been due either to reduced GA content, due to CCC treatment of the plants prior to anthesis, or the presence of CCC in the seeds. The seeds were therefore extracted and tested for CCC. Approximately 100µg of CCC was present per seed, as shown by biological and chemical methods. Thus, CCC accumulated in Pharbitis nil seeds and dwarfed the growth of progeny of CCC treated plants.

E. EFFECTS OF CCC ON CELL DIVISION AND MORPHOLOGY OF PLANTS.

CCC has a definite effect on the morphological development of plants. Treated plants appear to be shorter and more compact which is due to the shorter internode length. Stems of the plants are usually thicker than the stems from untreated ones. The plants also appear much darker in color (37,38,44,50). These symptoms were first reported by Tolbert and Wittwer, but their findings have been confirmed by many other workers in the past few years. CCC has been shown to have a variety of effects on different plants, and different varieties from the same species of plants have shown different responses.

Plants most sensitive to growth-retarding substances are those which grow slowly and constantly. Foliage plants, such as Philodendron, Diffenbachia and Peperonia grew without apparent response to even massive amounts of CCC.

Norris (28) observed different responses to CCC among different grass species. Short stemmed, bunch type grasses were affected less than the long stemmed rhizomatous types. Oats appeared to be less responsive to treatments with CCC than wheat and barley, while brome was the most sensitive of the forage species investigated.

A seasonal variation to CCC treatments has also been observed. The effects of CCC were much less in summer (3,4,5,37). Norris (28) also reported that the effect of CCC appeared to be greater in the winter than in the summer. He states that light intensity and the effect of CCC did not show significant interaction, and

suggests that the day length or photoperiod may be more critical in determining the magnitude of the effect of CCC than light intensity.

Wittwer and Tolbert (50,51) and Tiessen (40) reported that tomato plants developed thicker stems and darker green savoyed leaves. The growth changes were similar to those produced by exposure to high light intensities and low temperatures, and were opposite to, and more persistent than those induced by gibberellin.

Excessive amounts of CCC not only greatly suppressed growth but also caused a pronounced chlorosis (4,50). The chlorosis began along the midrib of the leaflets and extended towards the margins. In severe cases necrotic areas developed, followed by marginal burning. These symptoms usually developed from concentrations in excess of $10^{-4}$M in solution culture. The germination of seeds was delayed after CCC treatments (5), root development was initially inhibited and internode length was less on most plants (41). Leaves were a much darker green on treated than those on untreated plants.

CCC also promoted earlier tillering of the young wheat plants within a few days to two weeks after treatment (44). Norris (28) working with plants from the gramineae species also observed increased tiller production on plants treated with CCC in low dosages, but in dosages such as 20 to 40 lbs./acre the retardation was so high that it reduced tillering.

Wittwer and Tolbert (50) reported that the dry matter content

of tomato plants was increased by CCC treatment, more so for the roots than the tops, resulting in an increased root-to-shoot ratio. Fletcher and Renney (10) also reported a similar effect on tomatoes, beans and bent grass. The increased root top ratio might be due to a reduction in top growth without alteration of root growth.

Monselise and Halevy (15) confirmed that CCC treatment produced thicker leaves. They also showed that discs from bean leaves of CCC treated plants had a greater dry weight than those from the control. Untreated plants had a maximum leaf dry weight at midnight, whereas leaves of CCC treated plants attained maximum dry weight about one hour after sun-set, followed by a steady decline.

Humphries (19) postulated that mustard plants responded to CCC treatment by an increase in total leaf area, both in the greenhouse and under artificial fluorescent lights. In the greenhouse there were more leaves and in the growth chamber, larger leaves. The net assimilation rate fell, probably due to less growth and consequent lower demand for photosynthate.

Das (9) studied the growth regulator effect on top root ratio and the cation exchange capacity of snap beans and sweet corn. He used gibberellin, CCC and other growth regulators. CCC at 200 ppm concentration produced no significant effect on top root ratio, or root cation exchange capacity.

Anatomical observations reveal that differences in the length of the first internodes of control plants and those treated with CCC are due only to differences in cell number (5). CCC reduces

mitotic activity at least in the first internode. The application of gibberellin to CCC treated plants increased the length of the first internode by increasing the number of cells per internode. According to Zeevaart (53), available evidence suggests that growth retarding CCC inhibits flower formation in Pharbitis nil by inhibiting cell division in the plumule during the period when the floral stimulus is present.

Similar results were reported by Cathey and Stuart (5) concerning the inhibition of cell division.

F. EFFECTS OF CCC IN RELATION TO CROP YIELDS.

Experiments on maturation and crop yields were only suggestive, because few field trials have been attempted. Field tests have been disappointing because of the difficulty in supplying high enough concentrations of various chemicals to produce the desired affect on stem elongation. Eventually, treated plants grow as rapidly as untreated ones (4).

Tolbert (44) reported 2 to 4 days delay in heading out of wheat following treatment with CCC in greenhouse pot culture, although the yield was not affected. Cowley (Thomas's review 42) compared the effect of CCC and several other growth regulators, applied as foliar sprays to three varieties of cotton at the six leaf stage and at onset of flowering. In addition to retarding stem growth, the application resulted in some delay in maturity and reduction in yield, although boll weight was not affected. Thomas (42) also observed reduction in the rate of flowering in

cotton as a result of CCC treatment, with severe reduction in boll set and cotton seed production.

Tiessen (38) treated muskmelons at different growth stages with GA and CCC. The chemicals were applied to banded plants in the greenhouse both as a soil drench and foliar spray, and as a soil drench in the field at $10^{-3}$M solution. The CCC treatments increased yields by 27% (under glass, foliar), 23% (under glass soil drench) and 25% (field soil drench) when compared to control.

CCC was reported by several workers to increase early maturity of tomatoes (1,39,41,50,51). Andrew and Knowles in 1961 (1) treated tomatoes with CCC, $10^{-3}$M applied as a soil drench to plants in veneer bands, ten days before field setting; and in another treatment, $5 \times 10^{-3}$M at the time of field setting with starter solution. The application of CCC at field setting time had a greater effect than when applied 10 days before field setting. CCC increased the early yield almost 50% over control. The experiment was repeated in 1962 with various concentrations. The results indicated that the high concentrations were more effective than the low concentrations in dwarfing plants, but had no greater effect on flowering and fruiting.

Heeney (18) treated tomato plants with CCC in 10, 50 and 100 ppm concentrations using drench and foliar applications. He found that the soil drench treatment increased the blossom number without reduction of fruit set. Early yields were increased on an average of 5%. There was no measurable difference between various concentrations.

Tiessen (40) studied the effect of CCC on tomatoes and peppers at various temperatures. He also compared the difference between drench applications and foliar treatments. When the first true leaves appeared, half the tomato and peper plants were placed in a cold house (minimum night temperature 54° to 65°F) and the rest stayed in a warm house (minimum night temperature 64° and 68°F).

With peppers, the CCC drenches tended to suppress earlier fruit number and yield as a result, perhaps, of severe stunting of plants. The foliar treatments of CCC on peppers tended to stimulate a slightly earlier yield, and also increased fruit number, but this was more evident on total yields. None of the foliar treatments affected the fruit size of either the early or the total pepper yields, but yield was stimulated at warmer temperatures and at cooler temperatures an additional yield effect was evident. The CCC drenches reduced the total tomato yields by reducing the fruit number and size. The foliar applications of CCC on tomatoes in this experiment did not have any significant effect. However, in another experiment in 1962, Tiessen (39) had some encouraging results with foliar applications. Tomatoes were treated with foliar spray of 10 ppm CCC either as one application at the second to fourth true leaf stage, or two applications one week apart, the first being at the third to fourth true leaf stage. There was no significant difference in the yield of treated and untreated plants grown at 55°F, but plants treated with CCC as above, and grown at 65°F increased

yield over control and outyielded those grown at 55°F by 11 to 46%. This indicates that tomato plants treated with CCC at correct concentrations, growth stage and temperature, can increase early tomato yields.

In 1964 Tiessen and Rieger (41) soaked tomato seeds in CCC solution and water, and reported an increase in tomato yields as a response to the CCC treatment. Cucumber seeds were soaked in the same manner before field setting. Seeds soaked in water yielded 28% over yields obtained from dry seeds, but none of the CCC seed soaking treatments increased yields over the water soaking. They also treated cucumbers with CCC when the leaves were at the 1 1/2 to 2 inch diameter stage, and reported that the yield was 5 to 15% less than control, and the size of the vines was reduced to 30 to 44% over control. They suggested that, due to the smaller vine, the plant population could be increased 25 to 40%, and a yield increase of 20 to 30% could be anticipated.

They also reported an increase in bean yields as a result of CCC treatment at the 2nd trifoliate leaf stage. In addition to this, reduction in plant size was noted, when compared to control. These findings also indicate that rows could be spaced closer together, thereby increasing the plant population and thus increasing yields considerably.

Muskmelons were sprayed with CCC in the greenhouse, when the plants were at the 1st to 2nd and 3rd to 4th true leaf stage, and in the field about three weeks after field setting (41). All chemical treatments produced a slightly higher, but not significant

increase in yield over the control.

G. THE RELATIONSHIP OF CCC AND RELATED COMPOUNDS TO GIBBERELLIN AND AUXIN.

Leopold (21) agrees with other workers in stating that CCC strongly inhibits growth, and that its action is prevented by the application of gibberellin. The mutual antagonism is not restricted to growth affect. CCC can impose dormancy of lettuce seeds, an effect which can be overcome with gibberellin.

If growth inhibitors are acting competetively with gibberellin at a common site, one would hope that they might be structurally similar to the gibberellins in some respect. Since they are not, Tolbert (54) has suggested caution in considering them as true antigibberellins.

Kuraishi and Muir (20) studied the interaction of GA with CCC and phosfon in leaf tissue of Raphanus sativus. The inhibition of the effect of CCC and phosfon was greatest with the highest concentration of GA. The same effects were found when indoleacetic acid (IAA) was present.

Cytohistological studies with Chrysanthemum morifolium (5) have shown that retardants inhibit cell elongation and division in the subapical tissues of treated stems, and GA prevents the inhibition. Because of the limitations of application and transport of substances in intact plants, Sachs and Wohlers (30) used tissue explants cultured in vitro for continuing quantitative investigations of this physiological antagonism at cellular

level. They found that the three inhibitors of stem elongation, Amo-16, CCC and phosfon, inhibit cell division and expansion in tissues cultured in vitro. However, contrary to the case in intact plants, gibberellic acid does not prevent the retardant-induced inhibition in vitro. Supplementary auxin was also without effect in preventing the inhibition. Thus, the effect of the retardants cannot be simply that of inhibiting gibberellin or auxin synthesis. With respect to growth, carrot, chrysanthemums and geranium tissues were equally sensitive to all three retardants, whereas tobacco tissues are considerably more resistant to Amo and apparently unaffected by CCC.

Wittwer and Tolbert (51) studied the effects of CCC, GA and IAA on fruit setting of tomatoes. CCC used alone had no measurable effect on the growth of non-pollenated tomato ovaries, but in combination with $10^{-3}$ IAA and $10^{-5}$ M gibberellin, a synergistic growth rate was induced beyond that which could be ascribed to an IAA-gibberellin interaction. Two conditions have now been reported in which CCC and related compounds timulated parthenocarpic fruit development. Instances of increased vegetative and dry matter accumulation with the use of low levels of CCC and related compounds have been observed, where presumably auxin and gibberellin were not limiting. A combination of IAA, gibberellin and CCC had a synergistic effect upon parthenocarpic fruit development.

Halevy (13) offers an explanation for the CCC-gibberellin interaction. He used cucumber seedlings and measured the pero-

xide and IAA activity after treating plants with gibberellin or CCC and other growth retardants. Gibberellin decreased the activity of enzymes, resulting in a higher level of IAA in the plants, while CCC increased enzyme activity and reduced the level of IAA. GA and CCC applied simultaneously, nullified each others effect. From this work Halevy concluded that CCC exerts its effect on plants by interacting with gibberellin on IAA and peroxidase activity, and thus reduces auxin level in the tissue, resulting in the observed morphological changes. This statement is also supported by the results of Norris, who found a lower IAA content in CCC treated wheat seedlings.

The inhibitory effect of CCC on coleoptile growth was also overcome by higher concentration of IAA, but not by GA (20). Stem segments of peas, their growth retarded by CCC did not respond to treatment with GA, but had their length increased by as much as 3.3 times in IAA solution. The diffusible auxin from the stem apices of pea plants with growth retarded by CCC, was only one seventh as much as the diffusible auxin from normal plants. This indicates that the growth retarding effect of CCC is due to the lowering of diffusible auxin level in plants.

Paleg, Kende, Ninneman and Lang (29) observed the effects of GA on growth retardants on barley endosperm. CCC, Amo 1618, phosfon D, maleic hydroxide and two other growth retardants were not effective in retarding gibberellic-acid-induced reducing sugar released from barley endosperm. It is suggested that these compounds be termed "growth retardants" and not antigibberellins,

since they probably exert their effect as inhibitors of gibberellin biosynthesis.

Baldev and Lang (3) studied the control of flower formation by growth retardants and gibberellin in Salmolus parviflorus. The growth retardants Amo-16 and CCC inhibited flower formation and stem elongation in Salmolus parviflorus, a long day rosette plant. The vegetative growth of the plants was affected only slightly or not affected at all. Application of gibberellic acid completely reversed the inhibition of both flower formation and stem elongation caused by Amo. However, much larger amounts of GA were required to reverse the CCC inhibition of stem elongation, than that of flower formation.

These results strongly support the interpretation that the inhibition of flower induction in Salmolus by the growth retardant CCC, was the direct consequence of GA biosynthesis suppression in the plant. However, it was not clear whether CCC competes with GA for sites of action, by interfering with the biosynthesis of GA, or by the destruction or inactivation of GA. For this reason Harada and Lang (17) started an experiment which demonstrated that the fungus Fusarium moniliforme grown in the presence of CCC or Amo 1618 did not produce GA. It was concluded by Harada and Lang, that the effects of CCC on growth and development in higher plants can be overcome by the addition of gibberellin. The application of additional gibberellin counteracts the inhibition of gibberellin biosynthesis by CCC.

Starting out from these conclusions, Zeevaart in 1965 (55)

wrote that if CCC inhibits gibberellin production in cultures of Fusarium moniliforme, this suggests that CCC may exert its growth effects in higher plants by blocking GA production, causing GA deficiency, which would result in a dwarfed growth. This hypothesis was tested by using immature seeds of Japanese Morning Glory, Pharbitis nil, strain violet. These seeds are known to be a rich source of gibberellin-like substances. Pharbitis nil plants were grown in short days in a growth room at 25°C. CCC was applied via the roots at weekly intervals starting at anthesis. The immature seeds were harvested at different times after anthesis, and tested for gibberellin activity. The GA content of seeds from CCC treated plants were greatly reduced (up to 80%) as compared to seeds from untreated control plants. These results show that CCC inhibits GA production in higher plants.

## H. THE ECONOMIC IMPORTANCE OF CCC.

Authors working with CCC have suggested several economical advantages and possibilities. However, to date CCC has only one commercial use and that is the control of the height of poinsettias. Scaling of the heights of other plants has been suggested (7), controlling the lodging of wheat (44), increase in the flowering of azaleas (35) and increase in the resistance of treated plants to pest and disease (32,46).

Tiessen stated that CCC treatments may have several possibilities in the case of drench applications. Tomato plants were stockier, and such plants could be field set at a closer spacing,

and consequently increase the yield per acre. These stockier plants may be more resistant to setbacks during the field setting operation. Also stockier tomato plants could facilitate mechanical harvesting for the processing industry (40,41).

Foliar application of CCC to peppers tended to increase the yield in a manner similar to that of cold vernalization temperature (40). This could prove important in warm areas where it is not possible to obtain low night temperatures for plant vernalization.

There is also a possibility that CCC treatment may be beneficial in increasing early yields in tomato and some other horticultural crops.

## III. EFFECTS OF CCC ON THE GROWTH AND DEVELOPMENT OF *Lycopersicon esculentum* L.

### A. FIELD EXPERIMENTS.

#### 1. Materials and Methods.

The variety Early Alberta was selected because it is one of the best field varieties in the Edmonton area.

CCC used in this experiment was a synthetic product of American Cyanamid Company, Agricultural Division, Princeton, N.Y. It is formulated with a water-miscible solvent containing 11.8% (by weight) active ingredients, and in this study was applied as a soil drench application.

This experiment was conducted at the Parkland field laboratory during the summer of 1965. The field laboratory is situated in the black soil area of Alberta, south of the University Campus.

The official classification of the soil is Malmo Silt Loam, which is an eluviated black soil, developed on Lacustrine material. It has a high water holding capacity of 5" of water per foot of soil, with a top soil of 12-14". The soil has a 7% organic matter and the salinity of the subsoil is less than .2%. It is practically free from stone and topography is relatively level. It is considered good-to-very good arable soil.

The district has a rainfall of approximately 16-18 inches per year and the mean frost free period is 100 days with extreme variations from about 50 to 150 days (33).

Soil analysis was made in the Spring from the plots. It

was found that the available nitrogen ($N_2$) was 54 lbs./acre and phosphorus (P) was 47 lbs./acre. Excessive quantities of potassium (K) were found, the available amount being 600 lbs/acre. The pH was 5.6 and the soil contained a medium amount of organic matter.

The tomato plants were seeded in a greenhouse, the seeding medium being vermiculite. After germination the seedlings were pricked out to a 3-2-1 soil mixture in 3 inch wood veneer bands. They were watered with 100 ml of starter solution per band at a concentration of 1 oz. 10-52-17/gallon.

When the plants reached the 4th to 5th true leaf stage and were about 12 cms. in height, they received the first application of CCC. The following concentrations were applied:

| Treatment | Concentrations |
|---|---|
| 1 | 0 ppm CCC |
| 2 | 250 ppm CCC |
| 3 | 500 ppm CCC |
| 4 | 750 ppm CCC |

Plants in treatments 2, 3 and 4 received 100 ml. CCC solution and the plants in treatment 1 received 100 ml. of distilled water. A few days later the plants were transferred to the field laboratory and placed in a cold frame to harden. Ten days after the first CCC application, the plants were set in the field. At this time the second applications were made in the same concentrations as above.

Each plant received 1 litre starter solution containing CCC;

12 ozs. of 10-52-17 was dissolved in 12 gallons of water and then made up to the appropriate concentrations with CCC.

The experiment was designed as a randomized block with the four treatments replicated four times. Each treatment within each replication consisted of 10 plants per row. The plants were 1 m. apart in the row, and the rows were 2 m. apart. Guard rows were used on the borders of each replication.

At the time of the second application of CCC, the plants which had already received CCC treatments were somewhat darker green in color and shorter than the control.

The tomatoes received a fertilizer application two weeks after field setting time, at the rate of 300 lbs. 16-20-0/acre (34 grams per plant). This was put around the plant in a 30 cm. radius and was raked into the soil.

The following observations were made:

a) The heights of the plants were measured four times at weekly intervals, starting two weeks after the second CCC application.

b) The number of nodes preceeding the development of the first flower cluster was counted and also the number of flowers in the first flower cluster.

c) Yield data were recorded as weight of fruit in grams and number of fruit. The average weights of the fruits were also calculated, by dividing the weight of the fruits with the number of fruits.

2. Results.

Ten days after the first CCC application, plants appeared to be darker green in color, and stockier than untreated ones. This became more apparent after the second CCC application. Plants which received 750 ppm CCC had slightly savoyed leaves, while those which received 500 ppm and 250 ppm remained smaller throughout the growing season.

a) Effects of CCC on the height of tomato plants: CCC applications significantly reduced the heights of tomato plants compared to the control. The differences in height appeared ten days after the first application of CCC, but became more apparent two weeks after the second application. Plants which received 250 ppm CCC appeared similar to the control towards the end of the growing season, while the plants which received 500 and 750 ppm CCC remained somewhat shorter and smaller throughout the growing season. The differences in height are summarized in Table I and Figure 1.

b) Effects of CCC on the flowering of tomato plants: Plants which received high concentrations of CCC (500 and 750 ppm) produced the first flower cluster at a lower internode than the control. The flower number was also increased in the first flower cluster on plants which received CCC applications compared to control plants. The results are summarized in Table II.

c) Effects of CCC on tomato yields and average weights of tomato fruits: CCC applications significantly increased the early yield of fruit. Plants which received 250 and 500 ppm CCC had an early yield of approximately double the yield of the control

TABLE I

*Average heights in cms. of Lycopersicon esculentum L. (Early Alberta) two, three and four weeks after the second CCC application.*

| *Treatments* | *Mean Height* | | |
|---|---|---|---|
| | *2 weeks* | *3 weeks* | *4 weeks* |
| | *After the second CCC application* | | |
| 0 ppm CCC | 20cm a† | 34cm a | 44cm a |
| 250 ppm CCC | 15cm b | 28cm b | 37cm b |
| 500 ppm CCC | 14cm bc | 24cm bc | 34cm b |
| 750 ppm CCC | 13cm c | 22cm c | 31cm c |

*Analysis of variance for height measurements*

| | | *2 weeks* | | *3 weeks* | | *4 weeks* | |
|---|---|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 2.66 | | | | 1.00 | |
| Treatments | 3 | 34.33 | 23.83** | 110.00 | 15.98** | 112.00 | 38.77** |
| Error | 9 | 1.44 | | 6.88 | | 2.88 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 1

The effects of CCC on the height of field grown Lycopersicon esculentum L. (Early Alberta).

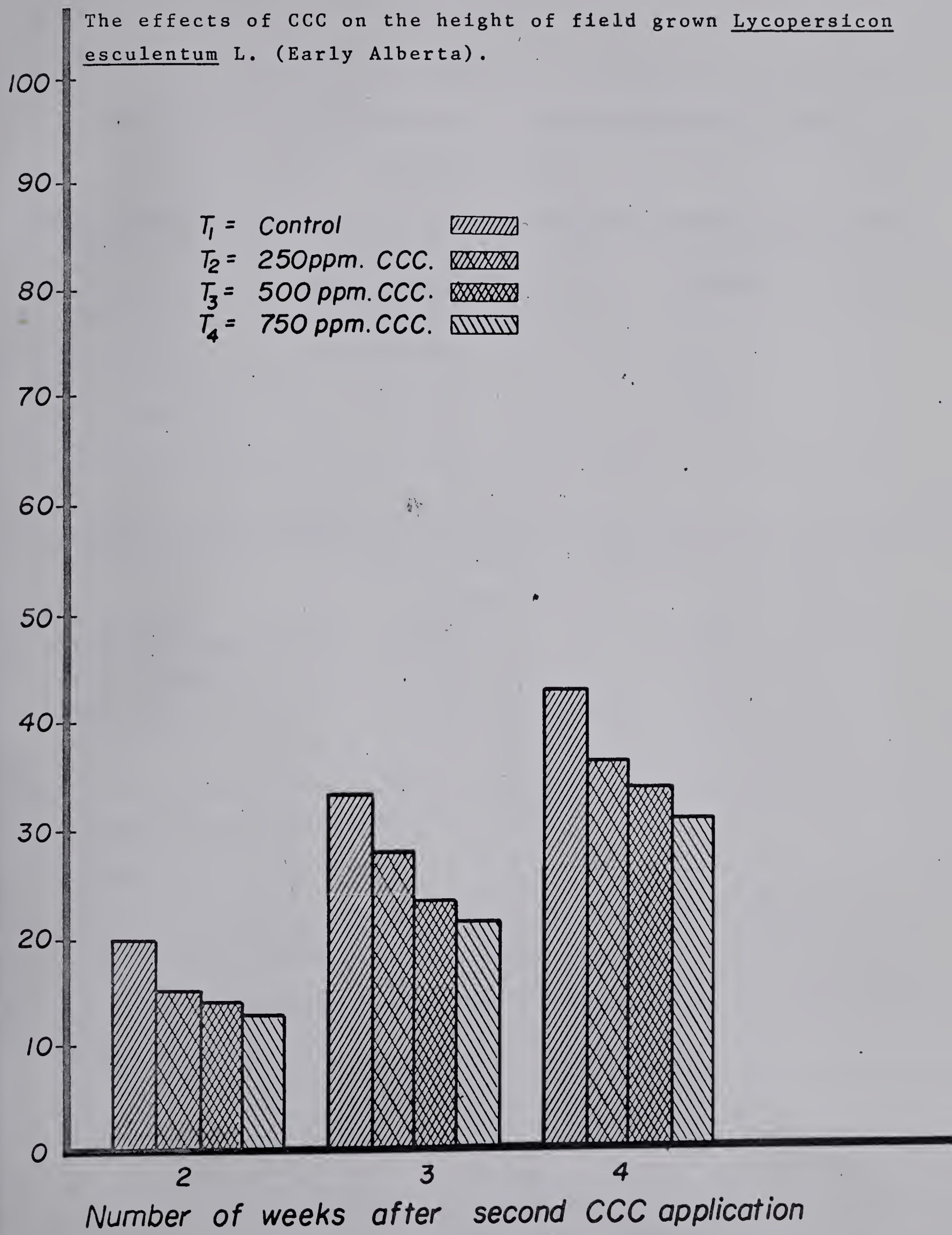


TABLE II

*Effects of CCC on the flowering of Lycopersicon esculentum L. (Early Alberta)*

| *Treatments* | *No. of nodes to first flower cluster* | *No. of flowers in first cluster* |
|---|---|---|
| 0 ppm CCC | 7.07 a† | 6.02 a |
| 250 ppm CCC | 6.68 ab | 6.75 b |
| 500 ppm CCC | 6.35 bc | 6.90 b |
| 750 ppm CCC | 5.85 c | 7.10 b |

*Analysis of variance table*

| | | *No. of nodes to first flower cluster* | | *No. of flowers in first cluster* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | .09 | | .166 | |
| Treatments | 3 | 1.03 | 11.4** | .866 | 5.18* |
| Error | 9 | .09 | | .167 | |

* Significant at 5% level.

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

plants, while the early yield of those receiving 750 ppm CCC was increased about 9% over control.

The average weights of the fruits were not affected by the CCC applications. The results are summarized in Table III and Figure 2.

TABLE III

*Effects of CCC on yield and average weight of fruits of* Lycopersicon esculentum *L. (Early Alberta).*

| *Treatments* | *Av. yield in kg.* | *Av. yield in no.* | *Average weight of fruits in grams* |
|---|---|---|---|
| 0 ppm CCC | 5.55 a† | 73.00 a | 72.25 a |
| 250 ppm CCC | 11.17 b | 134.00 b | 85.75 a |
| 500 ppm CCC | 10.98 b | 129.00 b | 85.25 a |
| 750 ppm CCC | 5.95 a | 84.00 a | 74.00 a |

*Analysis of variance table*

| *Source* | *d.f.* | *Av. yield in kg. MS* | *F* | *Av. yield in no. MS* | *F* | *Average weight of fruits in grams MS* | *F* |
|---|---|---|---|---|---|---|---|
| Replications | 3 | 196,730 | 2.76 | 97.66 | 5.49* | 233.00 | 2.87 |
| Treatments | 3 | 2,372,101 | 33.38** | 24.00 | 13.50** | 113.00 | 1.39 |
| Error | 9 | 71,053 | | 17.77 | | 81.00 | |

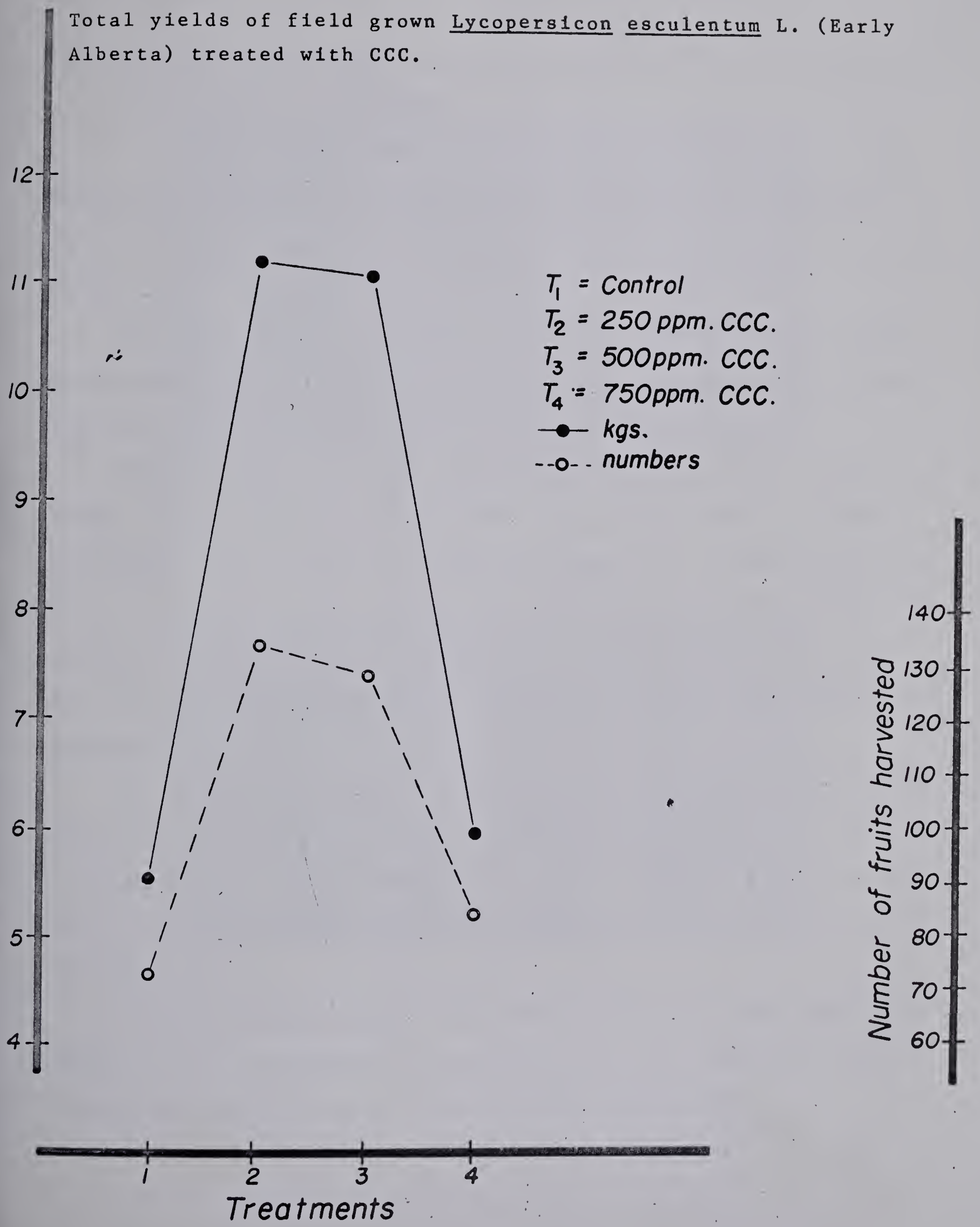
* Significant at 5% level.

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 2

Total yields of field grown Lycopersicon esculentum L. (Early Alberta) treated with CCC.



B. GREENHOUSE EXPERIMENTS.

1. Materials and Methods.

The forcing variety, Tuck Queen, was selected in this experiment because it is one of the best greenhouse varieties in the Edmonton district. CCC came from the same source as described in the field experiments.

The experiments were conducted at the University of Alberta greenhouse, in a 24 inch high ground bench in 3:2:1 soil mixture. The size of the bench was 42 ft. x 9 ft.

Soil analysis was made before the plants were set in the bench. The available nitrate was 2 ppm, phosphorus 4 ppm and potassium 8 ppm, which were all considered low in comparison with the level of 20-40 ppm nitrate, 5-10 ppm phosphorus and 20-30 ppm potassium, considered to be adequate in good soil. The pH was 6.3 and the soil contained 0.8 soluble salts which was considered negligible.

The plants were seeded in vermiculite and after germination they were pricked out in to a 1:1:1 soil mixture in 3 1/2 inch clay pots. They were watered with 100 ml. starter solution per pot. The concentration of the solution was 1 oz. 10-52-17/gallon of water.

When the plants reached the 4th to 5th leaf stage, and were about 12 cms. in height (Illustration #1), the first CCC applications were applied in the following concentrations:

Illustration 1.


Tomato plants at the time of the first CCC application.†

Illustration 2.


Tomato plants at the time of the second CCC application (Ten days after Illustration 1).†

†$T_1$ = 0 ppm CCC
$T_2$ = 150 ppm CCC } soil drench
$T_3$ = 500 ppm CCC
$T_4$ = 150 ppm CCC } spray
$T_5$ = 500 ppm CCC

| Treatment | Concentrations | |
|---|---|---|
| 1 | 0 ppm CCC | |
| 2 | 150 ppm CCC | } soil drench |
| 3 | 500 ppm CCC | |
| 4 | 150 ppm CCC | } spray |
| 5 | 500 ppm CCC | |

Plants treated with a soil drench received 100 ml. CCC per pot, and those in $T_4$ and $T_5$ were sprayed with a jet-type hand sprayer until the chemical was running off the leaves and stems. A few drops of Tween 20 were added to the spray solution to increase the adhesion to the plant.

Ten days after the first CCC application the plants were set in the bench and received the second CCC treatment. The concentrations were the same as above. Plants treated with CCC as a soil drench received the CCC with the starter solution. Ten ounces of 10-52-17 was dissolved in 10 gallons of water then made up to the appropriate concentration with CCC. Plants treated with the CCC foliar application received only starter solution.

The experiment was designed as a randomized block with the five treatments being replicated three times. Each treatment within each replication consisted of 5 plants per row. The plants were 55 cm. apart in the row, and the rows were 75 cm. apart. Guard rows were used along the two ends of the bench.

The tomatoes received fertilizer applications at weekly intervals. During the first and second weeks they received 10-52-17, and in the subsequent weeks 20-20-20. In all treatments 10

ounces of fertilizer were dissolved in 10 gallons of water and each plant received 500 ml. in a groove around the plant.

The following observations were made:

a) The heights of the plants were measured six times at weekly intervals, starting one week after the second CCC application.

b) Stem thickness and internode length from the 2nd to 7th node were measured.

c) The number of days from germination to the first inflorescence was noted, and also the flower position and flower number in the first inflorescence.

d) Yield measurements were taken in grams and numbers. The average weight of the fruits was also calculated.

e) In the last three harvests, the fruits were halved to determine the percentage of parthenocarpic fruits.

2. Results.

Ten days after the first CCC application, plants which received the drench appeared to be darker green in color and stockier (Illustration #2). These differences became more apparent a week after the second CCC treatment. By this time, plants which had received 500 ppm spray application were showing a slight chlorosis along the margin of the leaflets (Illustration #3). This disappeared about a week later. The color of the leaf was also darker green and somewhat smaller than the control.

Illustration 3.


Chlorosis on tomato leaves caused by application of CCC. $T_1$ control. $T_5$ received 500 ppm CCC as a foliar application.

Illustration 4.


Effects of CCC on the development of tomato plants. Treated plant has shorter internodes and the stem appears to be thicker. $T_1$ control. $T_3$ received 500 ppm CCC as a soil drench.

a) Effects of CCC on the height of tomato plants: Plants which received CCC as a soil drench and foliar application in 500 ppm concentration were significantly reduced in height compared to the control plants, and those receiving the 150 ppm foliar application. Four weeks after the second CCC treatment, the plants which received the 500 ppm foliar application had almost reached the same height as those which received the 150 ppm CCC foliar application. In all treatments, as the CCC concentrations increased, the height of the plants decreased. This difference was quite pronounced in the first 6 weeks after the second CCC treatment. Later, the heights of the plants levelled off, with the exception of those receiving the 500 ppm soil drench. The height differences are summarized in Table IV and Figure 3.

b) Effects of CCC on stem thickness and internode length: As a result of CCC treatment the stems were shorter. The shortest internode lengths were observed on the plants which received the CCC as a soil drench. The spray treated ones also appeared shorter but their differences were not significant (Illustration #4). Thicker stems were observed on the plants which received the CCC applications, especially those receiving CCC as a soil drench. The differences in stem thickness and internode length are demonstrated in Table V.

c) Effects of CCC on the flowering date, flower position and flower number in the first inflorescence: CCC treated plants flowered earlier and in most cases at a lower node than the non-treated control plants. Plants which received the 150 ppm foliar

TABLE IV

*Average heights of plants of Lycopersicon esculentum L. (Tuck Queen) in cms., two, four and six weeks after the second CCC application.*

| Treatments | Mean Height 2 weeks | 4 weeks | 6 weeks |
|---|---|---|---|
| | *After the second CCC application* | | |
| 0 ppm CCC | 32 a† | 52.00 a | 75 a |
| 150 ppm CCC } drench | 23 b | 38.00 bc | 62 b |
| 500 ppm CCC } drench | 22 b | 36.00 c | 58 c |
| 150 ppm CCC } spray | 30 a | 48.00 a | 68 d |
| 500 ppm CCC } spray | 22 b | 42.00 b | 64 c |

*Analysis of variance table*

| | | 2 weeks | | 4 weeks | | 6 weeks | |
|---|---|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* | *MS* | *F* |
| Replications | 2 | .50 | | 1.45 | | 36.00 | 9.94** |
| Treatments | 4 | 67.00 | 33.50** | 55.65 | 11.47** | 131.00 | 36.18** |
| Error | 8 | 2.00 | | 4.85 | | 3.62 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 3.

The effects of CCC on the height of Lycopersicon esculentum L. (Tuck Queen) grown under glass during the winter of 1965/66.

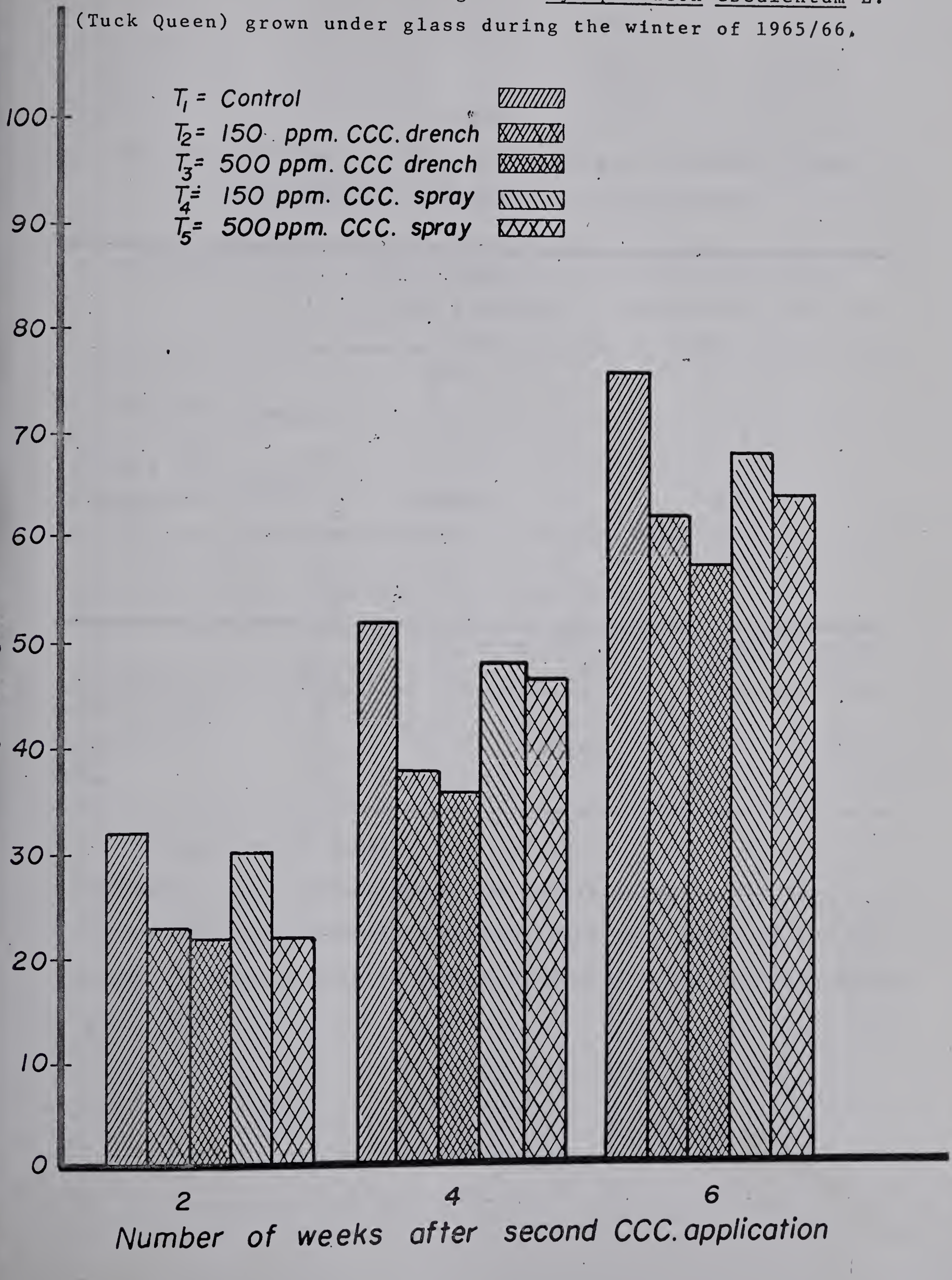


TABLE V

*Effects of CCC on the stem thickness and internode length of Lycopersicon esculentum L. (Tuck Queen).*

| *Treatments* | *Stem thickness at 6th internode in mm.* | *Internode length between 5th and 6th node in mm.* |
|---|---|---|
| 0 ppm CCC | 14.0 a† | 4.1 a |
| 150 ppm CCC } drench | 16.6 b | 2.6 b |
| 500 ppm CCC | 17.9 c | 2.7 b |
| 150 ppm CCC } spray | 15.3 d | 3.6 a |
| 500 ppm CCC | 15.4 d | 3.8 a |

*Analysis of variance table*

| | | *Stem thickness* | | *Internode length* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 2 | .45 | | 0 | |
| Treatments | 4 | 6.72 | 49.81 | 1.40 | 5.00* |
| Error | 8 | .14 | | .28 | |

* Significant at 5% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

spray flowered 5 days earlier than the control, while the soil drench also enhanced flowering, but to a lesser degree.

The position of the first flower cluster was significantly lowered on plants which received CCC as a soil drench or 500 ppm foliar application.

The number of flowers in the first flower cluster was also increased on plants which received CCC applications. The number of days from germination to the first inflorescences are summarized in Table VI and Figure 4. The flower position and flower numbers are summarized in Table VII.

d) Effects of CCC on yield and average fruit weights: In some cases CCC increased yield tremendously. In the first two weeks of harvest, the plants which received 150 ppm foliar application outyielded control about four times. The two drench applications also increased the early yield about 20% over control, while the 500 ppm foliar application reduced the yield 30% compared to the control. These results are recorded in Table VIII.

The total yields showed somewhat different results. Plants which received 150 ppm foliar application outyielded the control over 20%, while the 150 ppm soil drench and 500 ppm foliar applications were not significantly different from control. Plants receiving 500 ppm soil drench yielded 10% less than control. These results are summarized in Table IX. The average weight of the fruits was not affected by the CCC treatments.

e) Effects of CCC on parthenocarpic fruit development: It appeared from this experiment, that CCC increased the number of

parthenocarpic fruit. The percentage was rather high in all cases, especially plants which received the foliar applications and plants treated with 500 ppm CCC as a soil drench. The results are demonstrated in Illustration #5, Table X and Figure 5.

TABLE VI

*Effects of CCC on the number of days from germination until the appearance of the first inflorescence of* Lycopersicon esculentum *L. (Tuck Queen)*

| *Treatments* | | *Average days* |
|---|---|---|
| 0 ppm CCC | | 67.8 a† |
| 150 ppm CCC | } drench | 66.4 ab |
| 500 ppm CCC | | 65.2 b |
| 150 ppm CCC | } spray | 62.1 c |
| 500 ppm CCC | | 67.7 a |

*Analysis of variance table*

| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 2 | 2.50 | |
| Treatments | 4 | 16.00 | 16.00** |
| Error | 8 | 1.00 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 4.

Number of days from germination until the appearance of the first inflorescence of Lycopersicon esculentum L. (Tuck Queen).

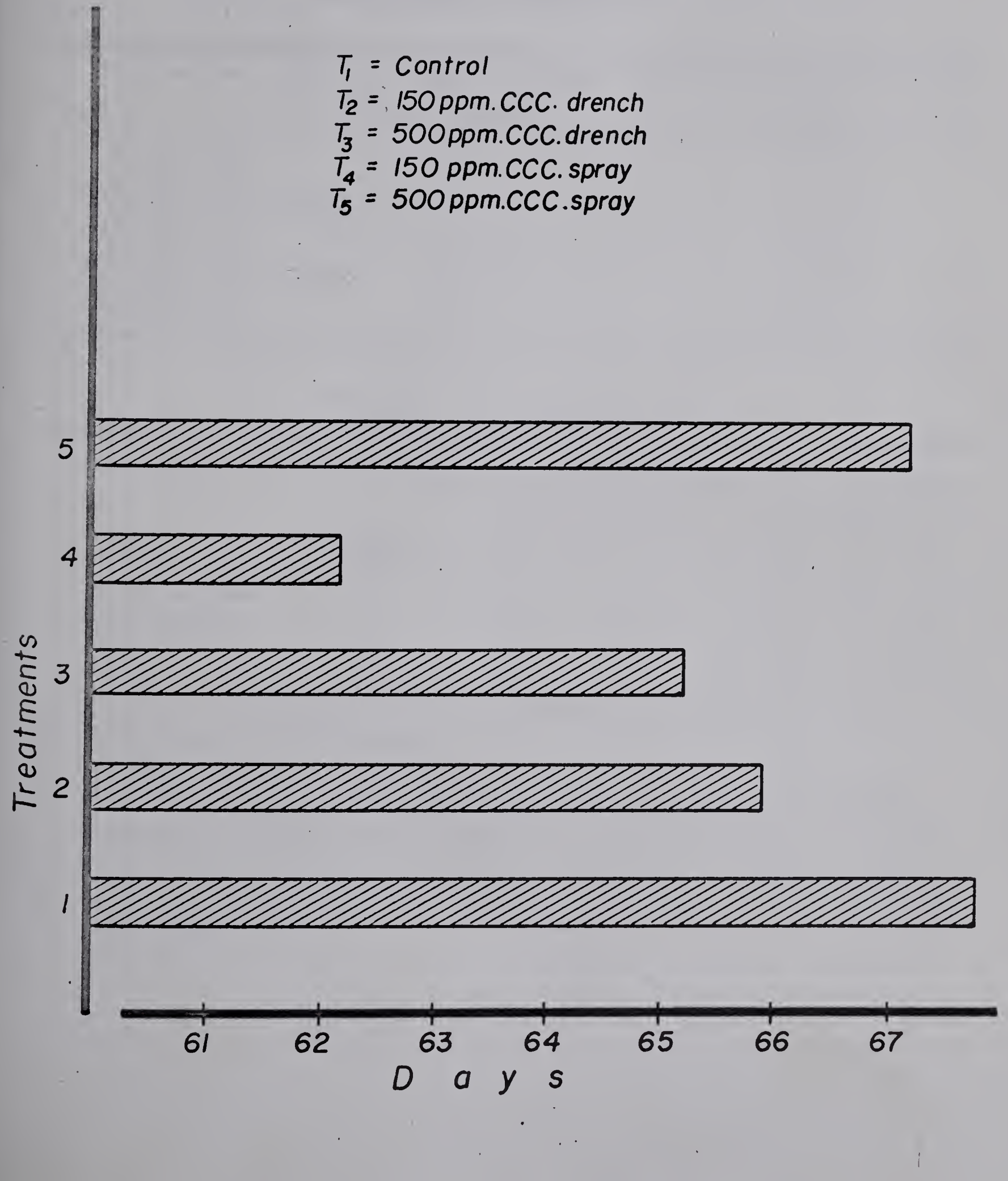


TABLE VII

*Effects of CCC on the flower position and number of flowers of Lycopersicon esculentum L. (Tuck Queen).*

| *Treatments* | *No. of nodes to first flower cluster* | *No. of flowers in first cluster* |
|---|---|---|
| 0 ppm CCC | 7.60 a | 8.1 a† |
| 150 ppm CCC } drench | 7.13 b | 12.3 b |
| 500 ppm CCC } drench | 7.13 b | 10.2 c |
| 150 ppm CCC } spray | 7.20 ab | 9.9 ac |
| 500 ppm CCC } spray | 7.13 b | 11.0 b |

*Analysis of variance table*

| | | *No. of nodes to first flower cluster* | | *No. of flowers in first cluster* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 2 | .02 | | 3.0 | |
| Treatments | 4 | .16 | 3.2 | 7.2 | 4.5* |
| Error | 8 | .05 | | 1.6 | |

* Significant at 5% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE VIII

*Effects of CCC on the early yields of Lycopersicon esculentum L. (Tuck Queen)*

| *Treatments* | *Av. yields in gms. Nov 2-16* | *Av. yields in no. Nov 2-16* |
|---|---|---|
| 0 ppm CCC | 382 a† | 4.3 a |
| 150 ppm CCC } drench | 460 b | 6.3 b |
| 500 ppm CCC } drench | 450 b | 4.6 ab |
| 150 ppm CCC } spray | 1,214 c | 11.6 b |
| 500 ppm CCC } spray | 280 a | 3.3 b |

*Analysis of variance table*

| *Source* | *d.f.* | *Av. yields in gms. MS* | *F* | *Av. yields in no. MS* | *F* |
|---|---|---|---|---|---|
| Replications | 2 | 5,314 | | 4 | |
| Treatments | 4 | 419,590 | 8.39 | 32.7 | 3.85* |
| Error | 8 | 50,000 | | 8.7 | |

* Significant at 5% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE IX

*Effects of CCC on total yields and average weights of fruits of Lycopersicon esculentum L. (Tuck Queen).*

| *Treatments* | *Av. yields in kg. Nov 2-Dec 3* | *Av. yields in no. Nov 2-Dec 3* | *Av. weight in grams Nov 2-Dec 3* |
|---|---|---|---|
| 0 ppm CCC | 2.23 a† | 31.0 ab | 72.3 a |
| 150 ppm CCC } drench | 2.28 a | 35.3 ab | 65.0 a |
| 500 ppm CCC } drench | 1.95 b | 27.3 a | 71.0 a |
| 150 ppm CCC } spray | 3.8 c | 43.0 b | 71.3 a |
| 500 ppm CCC } spray | 2.34 a | 35.0 ab | 66.6 a |

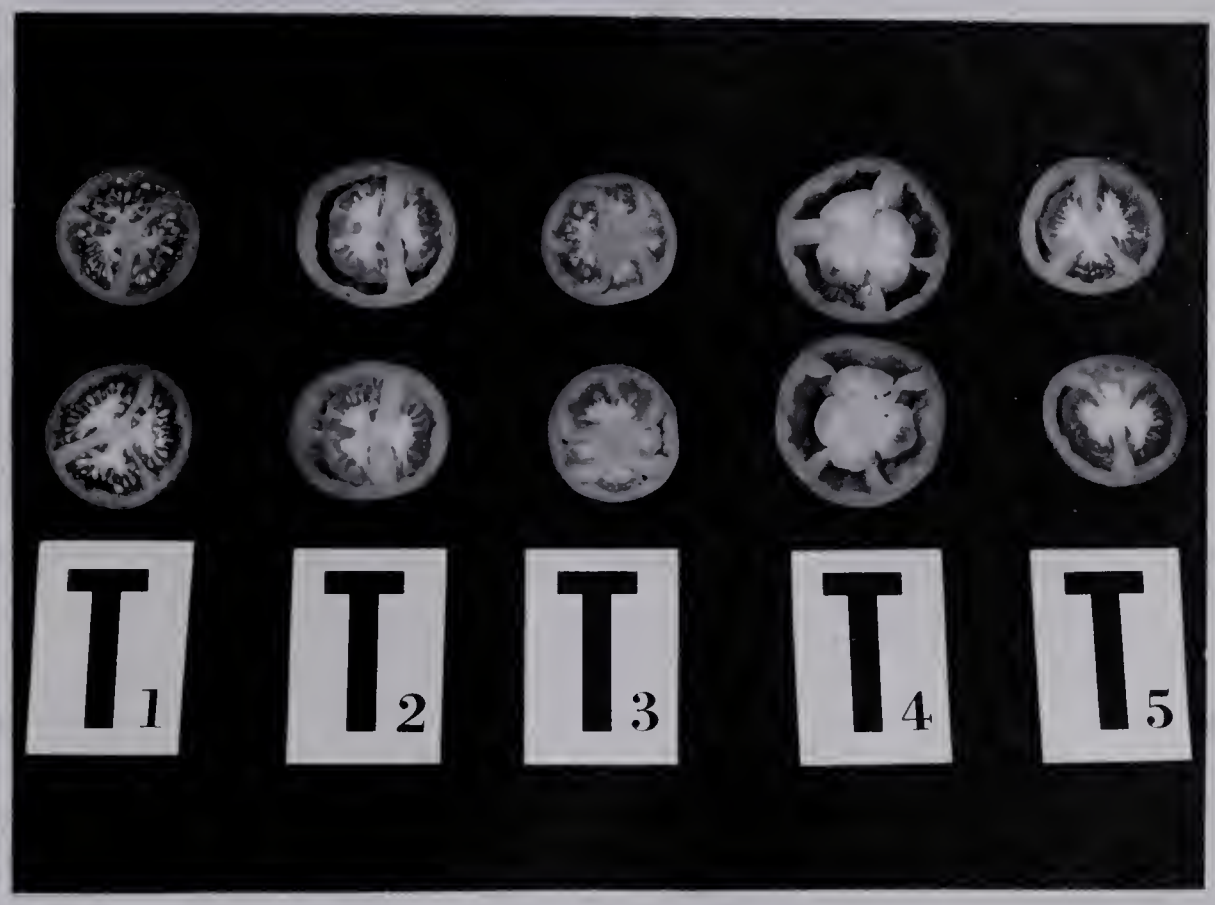
*Analysis of variance table*

| *Source* | *d.f.* | *Av. yields in kg. MS* | *F* | *Av. yields in no. MS* | *F* | *Av. weight in grams MS* | *F* |
|---|---|---|---|---|---|---|---|
| Replications | 2 | .39 | | 52.0 | | 84 | |
| Treatments | 4 | .52 | 4.00* | 102.5 | 2.47 | 31 | - |
| Error | 8 | .13 | | 41.50 | | 33 | |

* Significant at 5% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

Illustration 5.




Parthenocarpic tomato fruit. The highest percentage of parthenocarpic fruit was harvested from the plants sprayed 150 ppm CCC.

$T_1$ = 0 ppm CCC
$T_2$ = 150 ppm CCC
$T_3$ = 500 ppm CCC } soil drench
$T_4$ = 150 ppm CCC
$T_5$ = 500 ppm CCC } spray

TABLE X

*Effects of CCC on parthenocarpic fruit development of* *Lycopersicon esculentum* *L. (Tuck Queen).*

| *Treatments* | | *Mean Numbers in %* |
|---|---|---|
| 0 ppm CCC | | 25.30 a† |
| 150 ppm CCC | drench | 56.30 b |
| 500 ppm CCC | drench | 71.30 c |
| 150 ppm CCC | spray | 85.30 c |
| 500 ppm CCC | spray | 72.60 c |

*Analysis of variance table*

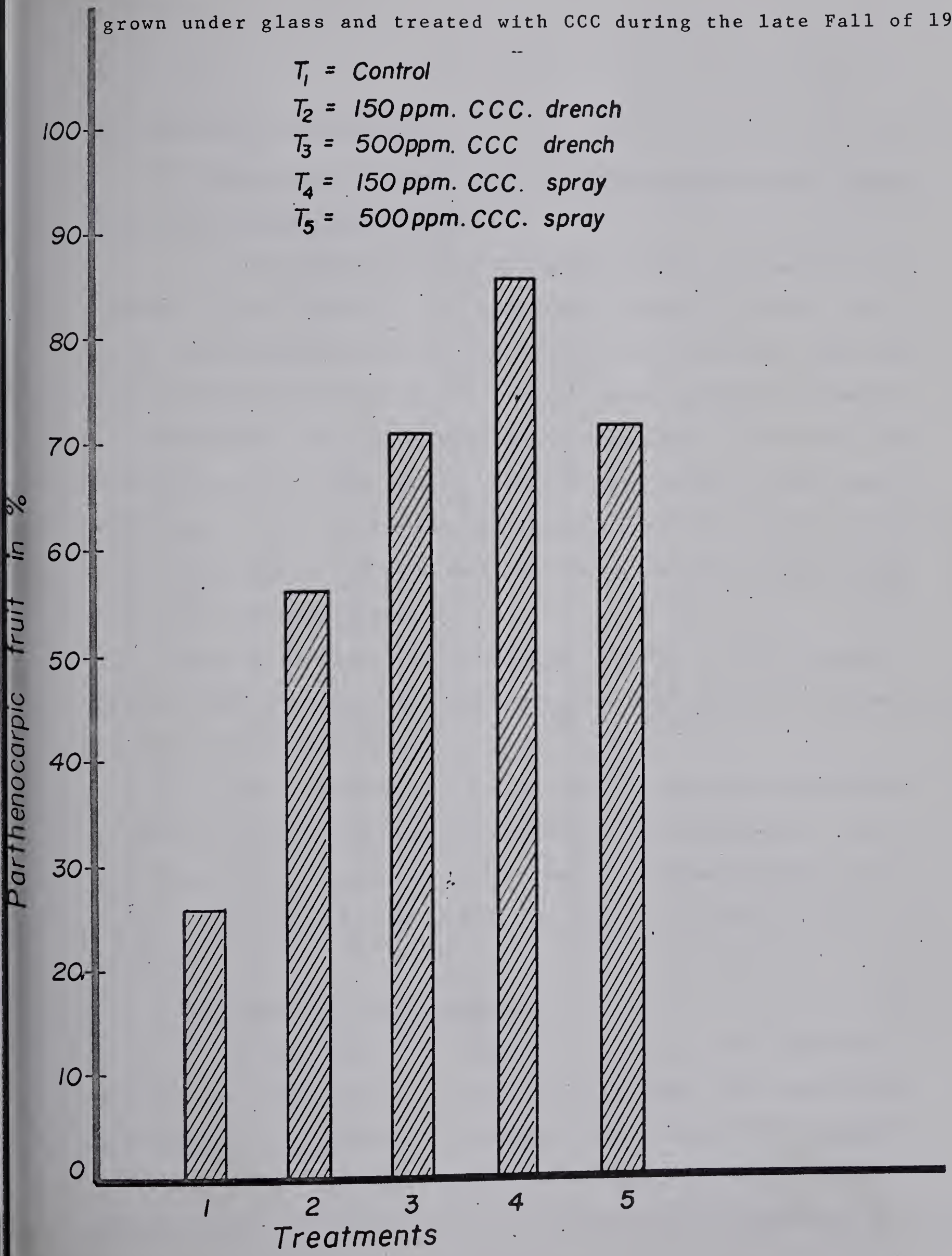
| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 2 | 83 | |
| Treatments | 4 | 1,591 | 23.74** |
| Error | 8 | 67 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 5

Parthenocarpic fruit of Lycopersicon esculentum L. (Tuck Queen) grown under glass and treated with CCC during the late Fall of 1965.



C. LABORATORY EXPERIMENTS.

1. Preparation of samples for the determination of chlorophyll and phosphorus content.

Leaf samples were collected from CCC treated and untreated tomato leaves at two different stages of growth. The first samples were taken at the 5th to 6th leaf stage when the plants were set in the bench. This was at the stage at which the plants received the second CCC application. The third leaf was removed from each plant. Thirty days later another set of leaf samples was collected. In this case the 6th leaf was removed from each plant. Only the leaflets from the leaves were saved. The rachis was discarded.

The soil particles were brushed from the leaflets before drying which was done in a forced air drying oven at a temperature of 65-70°C for 24 hours.

To ensure representative samples and complete extraction in analyses involving acetone extraction for chlorophyll, the dry tissues were ground to a fine powder before analyses. The samples were then stored in small aluminum containers until required.

2. Chlorophyll determinations.

a) Materials and Methods: The dry leaf tissue was weighed and then ground further using a mortar and pestle with fine sand as an abrasive. Complete extraction of the pigments was found to be dependent upon very thorough grinding. Three aliquots of approximately 10 mls. of 80% acetone were used to

extract the pigments from the ground sample. The extracts were filtered with a Buchner funnel through a double layer of Whatman #1 filter paper. The filtrate was washed with a further aliquot of acetone, then made upt to an appropriate volume and an aliquot used immediately to measure the pigment concentration.

The chlorophyll measurement was carried out with a Beckman DK-1 spectrophotometer. The optical density of the extracts was measured at 665 and 645 mμ, these being the respective peak absorption wavelengths for chlorophyll *a* and chlorophyll *b* in acetone.

The concentrations of chlorophyll *a* and *b* were calculated using the equations given by Maclachlan and Zalik (24). These were:

$$C_{\underline{a}} = \frac{(12.3 \times D_{663} - .86 \times D_{645})\ V}{d \times 1000 \times W}$$

$$C_{\underline{b}} = \frac{(19.3 \times D_{645} - 3.6 \times D_{663})\ V}{d \times 1000 \times W}$$

where: C = concentration in mg/g leaf tissue; *a* = chlorophyll *a*; *b* = chlorophyll *b*; D = optical density at wavelength given in mμ; V = volume of sample in ml.; d = path length of the cell in cm.; and W = weight of sample in grams.

b) Results: Samples taken 10 days after the first CCC application showed no significant difference in their chlorophyll content. Thirty days later there were significant differences between chlorophyll content of the samples. The chlorophyll content

was the highest in the plants which received the CCC as drench application and in the plants receiving the 500 ppm foliar application. The results are summarized in Table XI and Figure 6.

3. Phosphorus determinations.

a) Materials and Methods: The total phosphorus content of the leaf samples was determined by the Metavandate method. This procedure was developed on the basis of several other methods by the Department of Soil Science of the University of Alberta.

Ashing procedure: 1 gram of sample was weighed into a #2 crucible, then 5 ml. of magnesium nitrate solution was added from an automatic pipette and a little magnesium oxide powder was added on the end of a spatula. It was evaporated to dryness at a very low heat to prevent splatterings and frothings. The dried sample was then placed in a muffle furnace at 800°C for 30 minutes, The ash was then dissolved by 15 ml. of concentrated hydrochloric acid, and filtered into a 200 ml. volumetric flask.

Colorimetric procedure: 10 ml. aliquot was measured into a 50 ml. volumetric flask, 10 ml. combined $HNO_3$ Vanadate-Molybdate reagent and brought up to volume. The developed color was measured in a Universal Spectrophotometer at a wavelength of 415 mμ. Distilled water was used as a blank.

The concentrations of phosphorus were calculated using the following formula:

$$\%P = \frac{.8424(OD) - .05}{Wt.}$$

where: P = phosphorus in %; OD = optical density at 415 mμ

TABLE XI

*Chlorophyll (a+b) content of leaves of Lycopersicon esculentum L. (Tuck Queen) 30 days after the second CCC application*

| *Treatments* | *Mean Number mg/gram* |
|---|---|
| 0 ppm CCC | 6.95 a† |
| 150 ppm CCC } drench | 10.71 b |
| 500 ppm CCC | 11.62 c |
| 150 ppm CCC } spray | 8.50 d |
| 500 ppm CCC | 9.61 e |

*Analysis of variance table*

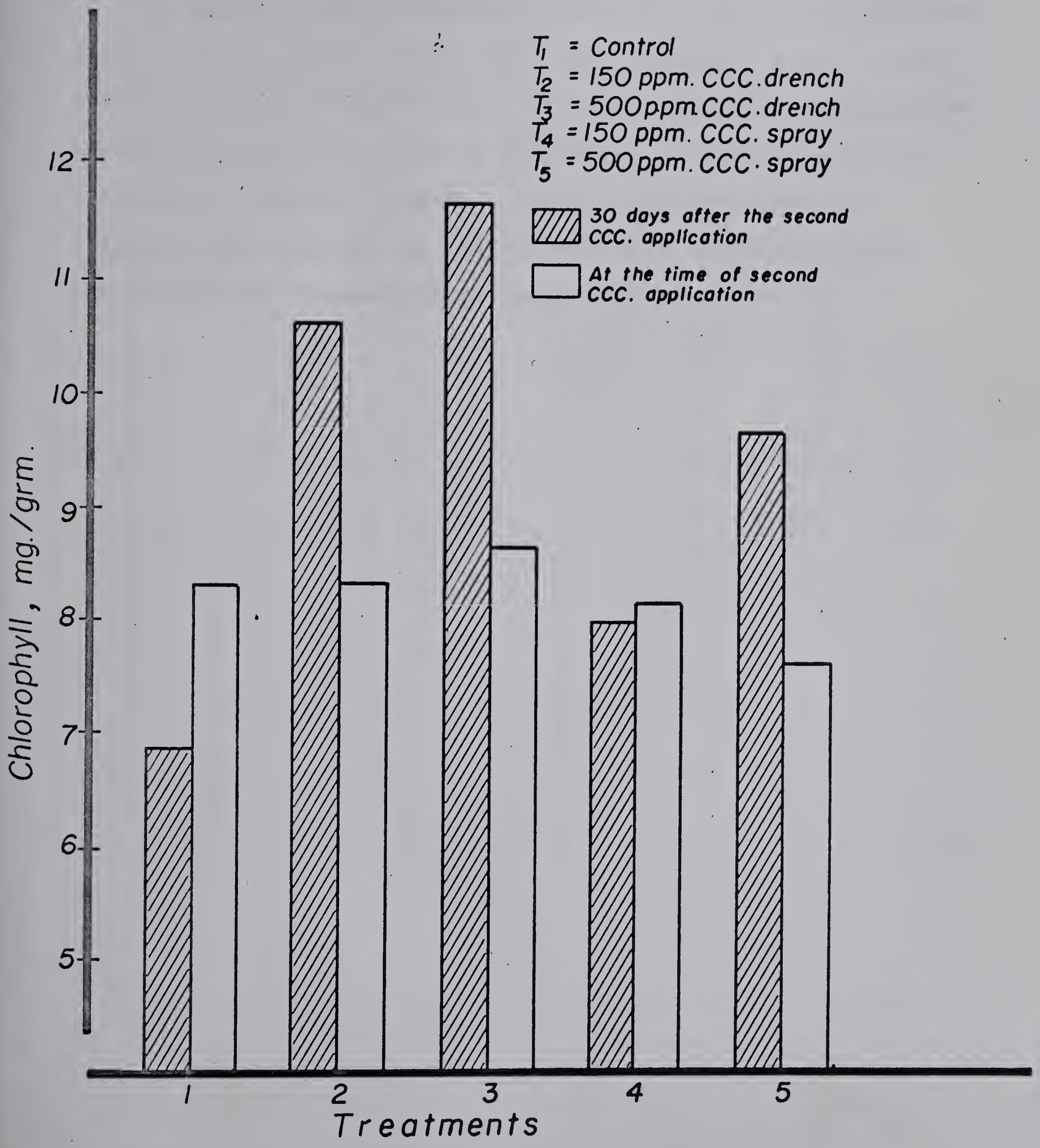
| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 2 | 0 | |
| Treatments | 4 | 10.00 | 40** |
| Error | 8 | .25 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 6.

Chlorophyll (a+b) content of Lycopersicon esculentum L. (Tuck Queen) leaves following two applications of CCC



wavelength; wt. = weight of sample.

b) Results: The phosphorus content of treated and untreated plants was not significantly different in either growth stage. However, samples taken 30 days after the second CCC application showed a slight difference in phosphorus content between CCC treated and untreated plants. The CCC treated plants had slightly lower phosphorus content than the untreated plants. The results are summarized in Table XII and Figure 7.

TABLE XII

*Phosphorus content of leaves of Lycopersicon esculentum L. (Tuck Queen) 30 days after the second CCC application.*

| *Treatments* | | *Mean Number in %* |
|---|---|---|
| 0 ppm CCC | | 1.30 a |
| 150 ppm CCC | drench | 1.09 a |
| 500 ppm CCC | drench | 1.08 a |
| 150 ppm CCC | spray | 1.04 a |
| 500 ppm CCC | spray | 1.07 a |

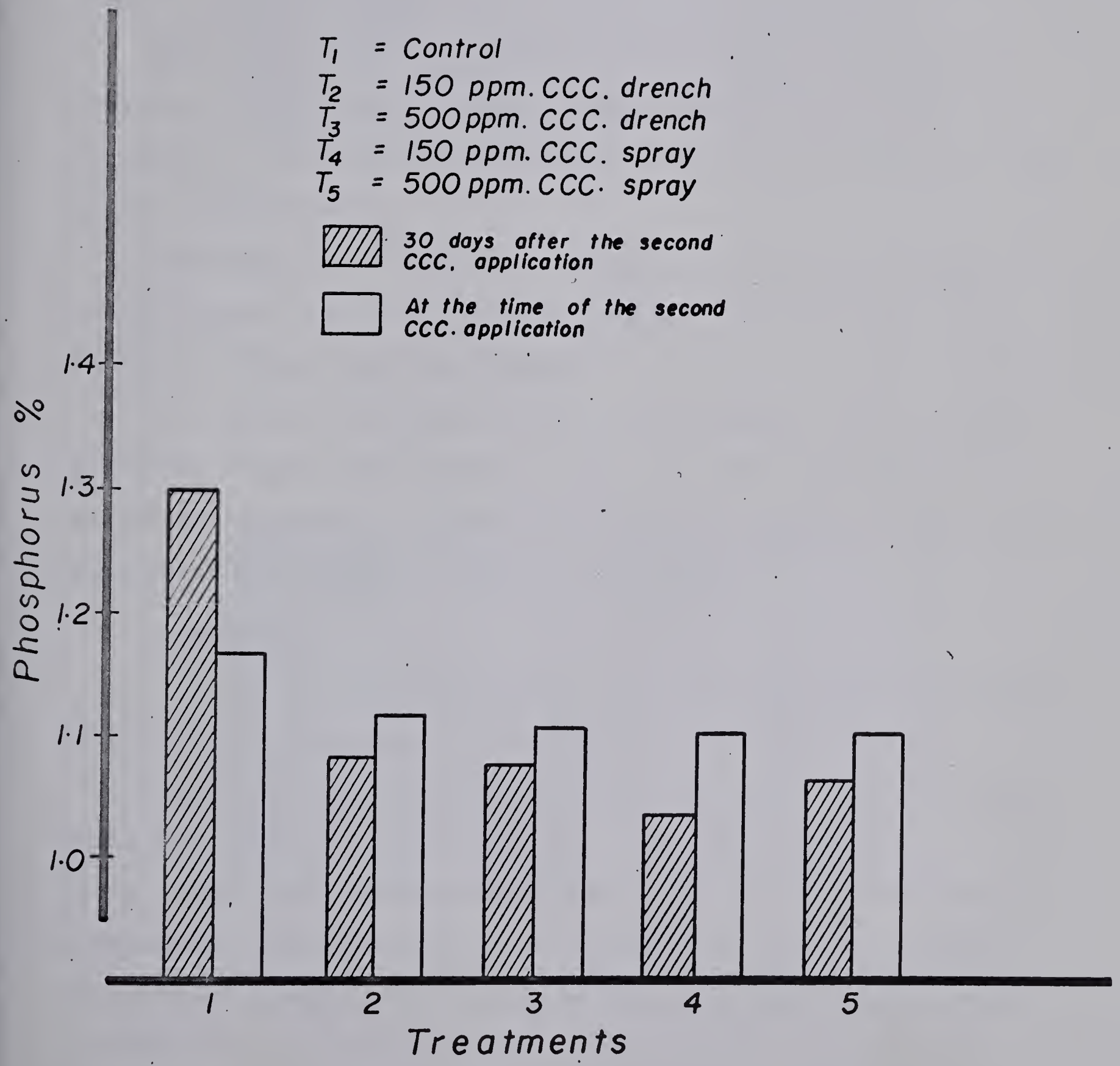
*Analysis of variance table*

| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 2 | .35 | |
| Treatments | 4 | 2.30 | - |
| Error | 8 | 1.67 | |

The phosphorus content of the leaves was not significantly different.

FIGURE 7.

Phosphorus content of Lycopersicon esculentum L. (Tuck Queen) leaves on dry weight basis.



D. GROWTH CHAMBER EXPERIMENTS.

1. Materials and Methods.

The previous experiments were repeated in the growth chamber using the same variety and the same treatments.

This experiment consisted of only 15 plants due to space shortage in the growth chamber. Three plants represented one treatment. The plants were planted in 1:1:1 soil mixture in one gallon plastic pails.

The objectives in this experiment were to counter-check the previously described results, and secondly to check the effect of CCC on root development.

The plants were grown in the growth chamber for six weeks after the second application of CCC. All the fruits were then harvested from all the plants, counted and weighed. The roots of each plant were removed, washed and weighed.

2. Results:

CCC had similar effects on plant growth and development as previously described in the first experiments.

The total fruit weight and number was the highest on plants which had received the 150 ppm CCC as a foliar application. The fruit number and weight was the lowest on the untreated control. These results are summarized in Illustration #6. The results were not analyzed statistically because the yield figures were obtained from only 15 plants.

The experiment also indicated that CCC reduces the size of

Illustration 6.

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| No. of fruits = | 22 | 36 | 29 | 30 | 26 |
| Weight in grams = | 72 | 1402 | 1328 | 1609 | 1099 |

Tomatoes harvested from three plants in each treatment six weeks after the second CCC application.

Illustration 7.


| $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|
| 120gm. | 98gm. | 70gm. | 104gm. | 106gm.† |

The effects of CCC treatments on tomato root development.

†Figures represent the average root weight of three plants.

the root. This reduction was the most severe in the case of the 500 ppm CCC drench application, followed by the 150 ppm soil drench and then the 150 ppm foliar treatment. It would appear that CCC had the least effect on the roots of plants when applied as a 500 ppm foliar spray. The results are demonstrated in Illustration #7.

E. DISCUSSIONS AND CONCLUSIONS.

The main purpose of these experiments was to investigate the effects of CCC on early yields of tomato plants under Alberta growing conditions.

It was reported previously (1,3,9,18,40,50) that CCC treatments increased early yields in tomatoes. Not every variety responded similarly to CCC, therefore, these investigations were initiated to determine how a local field and greenhouse variety would respond to CCC.

The experiments indicated that CCC applied either as a soil drench or as foliar treatments, increased the early yields. However, the 150 ppm CCC foliar treatment applied first at the 4 to 5 leaf stage and again ten days later, had the most beneficial effect. It increased the early yields in the first two weeks of harvest four times compared to control, and total yields from the first two clusters 20% compared to control. Similar results were obtained in the second experiment in the growth chamber (Illustration #6). Tiessen (39) also suggested that foliar applications of CCC might be beneficial at the right concentrations.

While 150 ppm CCC treatment increased early yield, 500 ppm CCC applied as a foliar spray decreased early yield 30% compared to control. The 500 ppm foliar spray was found to cause chlorosis on the margins of the leaflets, which disappeared within a week. Other workers (4,28,50) also reported chlorosis caused by excessive amounts of CCC along the midrib of the leaflets, which extended towards the margins. In the present experiment, however, it

started on the margins of the leaflets as shown in Illustration #6.

This may have been due to the fact that in previous reports chlorosis was induced by soil applications, while in this experiment it was caused by 500 ppm foliar application.

Soil drench applications approximately doubled early yields at 250 ppm and 500 ppm concentrations under field conditions with the variety Early Alberta. In the greenhouse, with the Tuck Queen variety, the 150 ppm and 500 ppm soil drench increased the early yield 20% compared to control. The 750 soil drench under field conditions and the 500 ppm in the greenhouse suppressed the growth excessively, which resulted in the reduction of total yields. Andrew (1) also noted increased early yield of about 50% under field conditions with $10^{-3}$M (about 158 ppm) CCC solution applied as a soil drench at transplanting time. Similar results were reported by Wittwer (50), Tiessen (39), and Heeney (18).

It was also reported that CCC applied as a soil drench increased the number of flowers in the first flower cluster, and that the first cluster appeared at a lower node (18,39,50). The present results confirm these reports. The first flower cluster appeared at a lower internode, and the number of flowers also increased in all plants treated with CCC compared to control. CCC also accelerated flowering. Plants which received the 150 ppm foliar sprays flowered five days earlier than control plants. Plants receiving other treatments also flowered somewhat earlier than the control plants. The above findings support those of Tiessen and Wittwer.

Tiessen (40) reported that CCC applied as a soil drench reduced the size of the tomato fruit. Reduction in size of the fruit was not reported in other reports. The average weights of the fruits in this experiment were not affected by CCC treatments either in the field or in the greenhouse.

The Tuck Queen tomato fruits grown in the greenhouse had a high percentage of parthenocarpic fruits. Previously only two cases were reported where CCC induced parthenocarpic fruit. According to Wittwer and Tolbert (51), when CCC, combined with GA and IAA was applied to tomato ovaries, parthenocarpic fruit was induced. They also reported that when CCC was applied at a low concentration to tomato plants with increased vegetative and dry matter accumulation, some parthenocarpic fruit was produced.

The experiment herein reported was conducted during the winter when the light intensity was very low, being 250 to 400 foot candle in the greenhouse. It is known that low light intensity is unfavorable to tomato fruit set, and this may have had an effect on the parthenocarpic fruit development. Another possibility may be that the tomato plants became infected with Cucumber mosaic virus (Marmor cucumeris) when the second cluster started to flower. This could have had some effect on the development of parthenocarpic fruit.

The percentage of parthenocarpic fruit was the highest (85%) from plants which received 150 ppm CCC as a foliar application. The control had 25.30%.

Fruits harvested from plants grown in the growth chamber had only a few parthenocarpic fruit, which would also indicate that in the greenhouse experiment there might have been an outside factor which increased the parthenocarpic tendency. It is felt that this should be further investigated.

CCC applied to the plants as a soil drench, suppressed plant growth considerably, especially the 750 ppm soil drench under field conditions and the 500 ppm in the greenhouse. However, all the soil drench applications retarded the heights of the plants significantly. These results supported the findings of other workers (1,18,39,40).

The foliar application had a slight effect on the size of the plants. The 500 ppm foliar treatment suppressed growth for about 10 days, but later the plants started to grow rapidly and almost equalled the height of the control.

The reduction in plant size was due to the reduction in internode length. The node number remained the same. The reduction of internode length was probably caused by the inhibition of cell division as suggested by Cathey and Stuart (6).

CCC applied as a soil drench also increased the thickness of the tomato stems, which was also reported by Wittwer (50).

The CCC treated plants had a darker color green than the control, as reported by several research workers (4,10,19,25, 28,37,38,44,50). Plants receiving the soil drench applications had the darkest color. The foliar application was not as effective on chlorophyll production as the soil drench.

Tolbert suggested that CCC treatments on barley seedlings altered the translocation of phosphorus. This appeared to be similar in tomato plants. The tomato plants treated with CCC had a lower percentage of phosphorus than the untreated control. These differences were not significant, but they indicate that CCC treatment may reduce the phosphorus content of tomato leaves.

The other possible explanation is that CCC treated plants were already in the advanced flowing stage, at which stage plants require more phosphorus. The fertility level of the soil was the same for CCC treated and untreated plants. Therefore, it is possible that phosphorus was translocated from the leaves of CCC treated tomato plants to the flowering parts.

It has been reported by several authors (4,10,50) that CCC treatment decreased the root development of the plant. These findings also support such reports. The plants which received 500 ppm CCC as a drench application exhibited the greatest retardation of roots. The foliar application of CCC had less effect on the root retardation.

It was suggested (11,14,27,52) that CCC treated plants might be able to withstand unfavorable environmental conditions better than the untreated ones. This was demonstrated about 6 weeks after field setting in a rain and wind storm. The CCC treated plants withstood the twisting affect of the wind, while the untreated ones were lying on the ground twisted and turned over.

From the practical point of view, it would appear that CCC treatments on tomatoes would have some possibilities commercially. Foliar application of 150 ppm CCC concentrations applied at the 4 to 5 leaf stage at first, and ten days later, resulted in the greatest increase in early yields. The foliar applications did not reduce the plant size to the same degree as the drench applications.

The drench applications of CCC proved to be more effective at lower concentrations, where the plants were not retarded so extensively. This was found with both field and greenhouse varieties. The other possible advantage of drench applications is that the transplants are stockier, and such plants could be field set at a closer spacing and increase the yields per acre. Also, the stockier plants may be more resistant to certain environmental conditions, and would result in a more rapid recovery in the spring. Smaller, stockier plants could also withstand wind damage much better than larger plants, and could facilitate mechanical harvesting for the processing industry.

# IV. EFFECTS OF CCC ON THE SEX EXPRESSION, GROWTH AND DEVELOPMENT OF *Cucumis sativus* L.

## A. FIELD EXPERIMENTS.

### 1. Materials and Methods.

The varieties Marketer, Hybrid Burpee and National Pickling were selected because these are important varieties under Edmonton growing conditions. The CCC came from the same source as described in the tomato field experiments.

The experiments were conducted at the Parkland field laboratory during the summer of 1965.

Soil analysis was made in the spring from the plots. The available nitrogen was 52 lbs./acre. The phosphorus was 41 lbs./acre of available phosphorus and excessive quantities of potassium were found, the available amount being 580 lbs./acre. The pH was 5.6 and the soil contained a medium amount of organic matter.

The cucumbers were seeded directly into the field. The experiment was designed as a randomized block with four treatments replicated four times. Each treatment within each replication consisted of 10 hills per row, seeded 1 m. apart in the row, and each row seeded 2 m. apart. Guard rows were used on the borders of each replication. The CCC treatments were as follows:

| Treatments | Concentrations |
|---|---|
| 1 | 0 ppm CCC |
| 2 | 500 ppm CCC seed treatment and soil drench |
| 3 | 500 ppm CCC soil drench |
| 4 | 1000 ppm CCC soil drench |

In treatment two, the seeds were soaked for 24 hours in 500 ppm CCC solution before seeding, and a soil drench was applied when the first true leaves were about 1 inch long. Plants in treatments three and four received a soil drench application when the first true leaf was about 1 inch long. This was applied in a groove around the hills at the rate of 1 litre per hill.

The cucumbers were fertilized with 300 lbs. of 16-20-0 per acre, two weeks after field setting time. The fertilizer was broadcast around the plant in a 30 cm. radius and was raked into the soil. The plants were thinned at the same time to 2 plants per hill.

The following observations were made:

a) The heights of the plants were measured three times at weekly intervals, starting one week after the CCC application.

b) The number of male and female flowers were counted twice per week.

c) Yield measurements were taken in grams and also in numbers.

2. Results.

In this experiment, CCC did not have an observable effect on the cucumber plants, and it is possible that the CCC was lost in the soil moisture before it could have been absorbed by the plants. The seed treatment also had no effect.

B. GREENHOUSE EXPERIMENTS.

1. Experiment No. 1.

a) Material and Methods: In this experiment the varieties Marketer and Hybrid Burpee were selected. The CCC came from the same source as described in the tomato field experiments.

The experiment was conducted at the University of Alberta greenhouse, in a 42ft x 3ft raised bench.

Soil analysis was made before the plants were set into the bench. The available nitrate was 35 ppm and phosphorus 12 ppm, while potassium was 7 ppm. The pH was 6.4 and the soil contained 2.2 soluble salts.

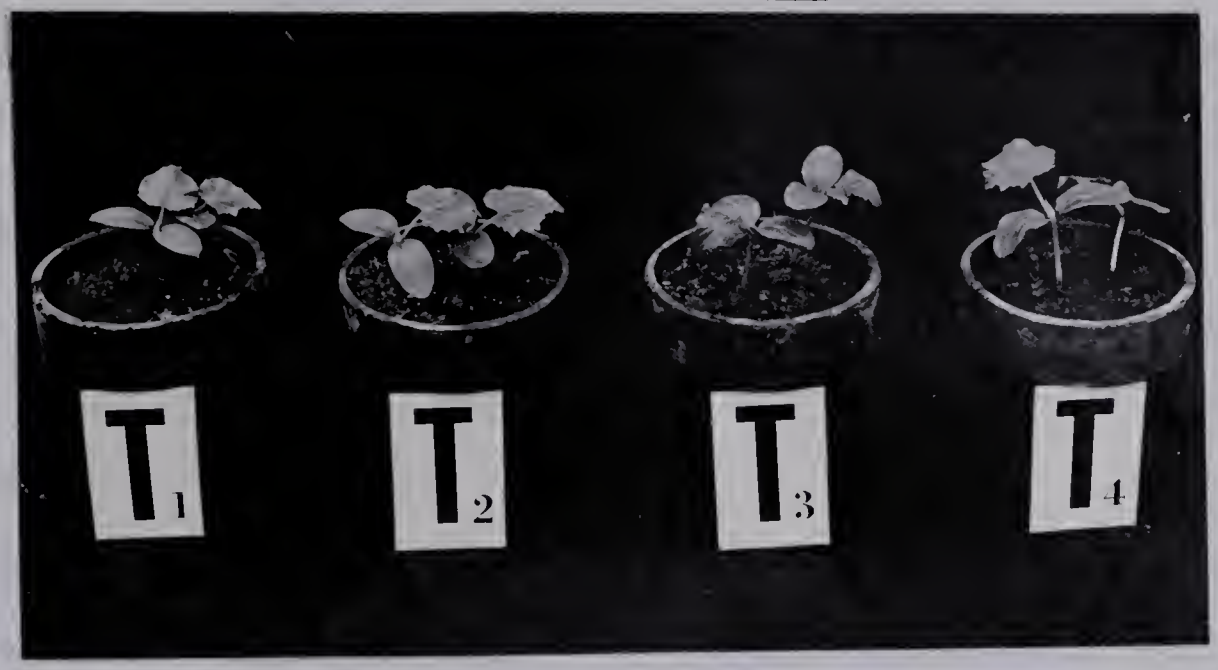
The seeds were placed in a U.C. mixture (50 sand: 50 peat) in 3" wooden veneer bands. They were watered with starter solution at a concentration of 1 oz. 10-52-17/gallon and 100 ml. of solution was applied to each band.

When the first true leaf was approximately 1 inch in length (Illustration #8), the first applications of CCC were made at the following concentrations:

| Treatment | Concentrations |
|---|---|
| 1 | 0 ppm CCC |
| 2 | 100 ppm CCC |
| 3 | 250 ppm CCC |
| 4 | 500 ppm CCC |

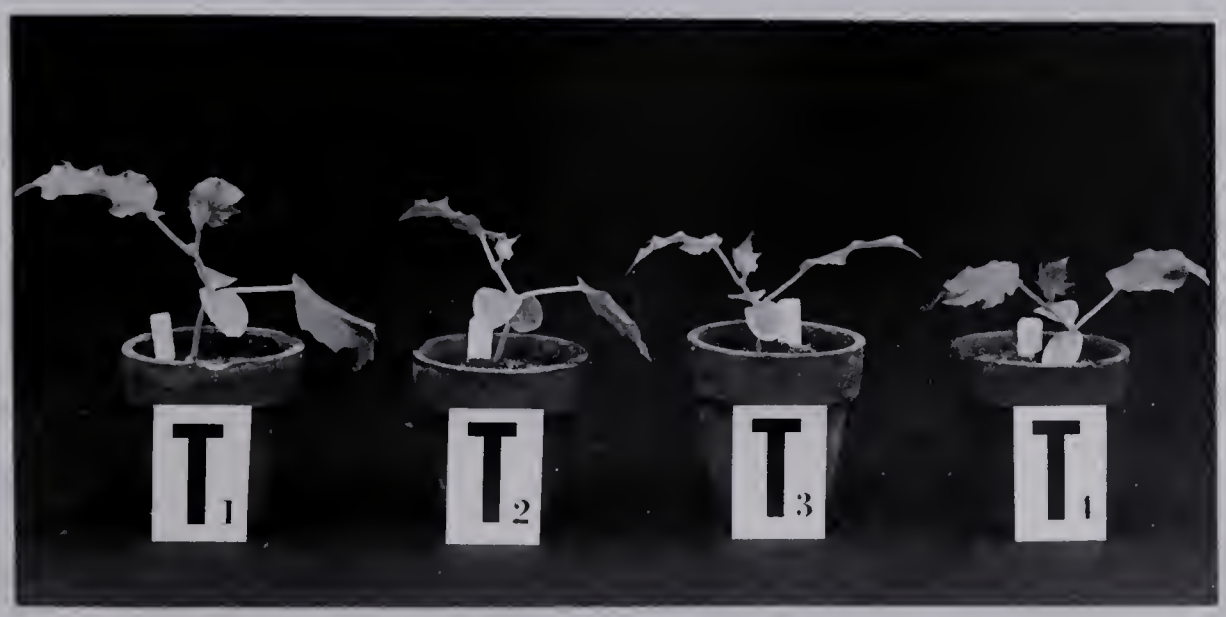
Plants received 100 ml. per band. Ten days after the first CCC application, the plants were set in the bench wehre they received the second CCC treatment (Illustration #9). The con-

Illustration 8.




Growth stage of cucumber plants at the time of first CCC application.†

Illustration 9.




Growth stage of cucumber plants at the time of second CCC application.†(Ten days after Illustration 8).

†$T_1$ = 0 ppm CCC
$T_2$ = 100 ppm CCC
$T_3$ = 250 ppm CCC
$T_4$ = 500 ppm CCC

centrations were the same as above. In this case the CCC was added to the starter solution (1 oz. 10-52-17/gallon) 500 ml. per plant.

The experiment was designed as a randomized block, with the four treatments replicated four times. Each variety was treated as a separate experiment. Each treatment within each replication consisted of two plants per hill and two hills per row. The hills were planted 50 cm. apart in the row and the rows were 35 cm. apart. Guard rows were used at each end of the bench.

Beginning one week after benching, the plants were trained upwards along a cord tied to a network of wires about 4 1/2 feet above the bench. Only one main stem was brought up to the top of the cord. When the branches started to grow at a leaf axil, they were pinched back at the second leaf. In this way the lateral branches also produced flowers and fruit.

Since the cucumber is a very heavy feeder, it was important to maintain a constant high level of nutrition. They received regular fertilizer applications in the first two weeks at the rate of 16 ozs. 10-52-17/1000 square feet at weekly intervals. During the next four weeks they received 15-30-15 at the rate of 16 ozs./1000 square feet, and during the remainder of the growing season 20-20-20 was applied at weekly intervals at the rate of 20 ozs./1000 square feet (12).

Sexual reproduction in the cucumber plant depends on pollen transference from the male flower to the female flower. Since there are normally no insects present in the greenhouse to

provide a means of pollen transfer a hive of honey bees was placed in the cucumber house. The day temperature was 80-90°F and the night temperature 70-75°F.

The following observations were made:

a) The heights of the plants were measured four times at weekly intervals, starting one week after the second CCC application.

b) The number of nodes preceeding the development of the first female flower were noted.

c) Male and female flowers were counted twice a week from June 4th to July 27th.

d) Yield measurements were recorded in grams and numbers. Fruits were picked when they were approximately 7-8 inches long and 2 to 2 1/2 inches in diameter. Misshapen or damaged fruits were removed as soon as it was evident that they would not develop normally.

b) Results.

i. Effects of CCC on the heights of cucumber plants: All plants which received CCC applications were significantly reduced in height compared to the control. This difference in height was first observed at the tine when the plants received the second CCC application (Illustration #9). The treated ones were shorter and the leaves were smaller than the control. The differences in plant size became even greater 10 days after the second CCC application.

CCC had a very strong retarding effect on all treated

plants, especially those which received the 500 ppm CCC. This effect was evident throughout the growing season, on both varieties.

The results pertaining to height measurements are summarized in Table XIII, Figure 8 and Illustrations #10 and 11 for Marketer, and in Table XIV for Hybrid Burpee.

ii. Effects of CCC on the node number preceeding the development of the first female flower: Treatments in most cases failed to result in female flower production at a lower node number. In the case of Hybrid Burpee, there was great variation within each treatment and the differences were not significant.

The Marketer variety showed a somewhat better response. The 100 ppm CCC treatment significantly reduced the node number to the first female flower compared to control. The other treatments, however, failed to show any significant difference compared to control. These figures are presented in Table XV for Hybrid Burpee, and in Table XVI and Figure 8 for Marketer.

iii. Effects of CCC on the sex expression of the cucumber: CCC treatments failed to increase the number of female flowers on the treated plants in either variety. Most treatments decreased the number of flowers compared to the control.

The number of male flowers, on the other hand, decreased on all cucumber plants treated with CCC. This decrease, in the case of the Marketer variety, was of sufficient magnitude to be statistically significant. In the case of the Hybrid

TABLE XIII

*Average heights of Cucumis sativus L. (Marketer) one, two and four weeks after the second CCC application.*

| *Treatments* | *Heights in cms.* | | |
|---|---|---|---|
| | *1 week* | *2 weeks* | *4 weeks* |
| | *After the second CCC application* | | |
| 0 ppm CCC | 30cm a† | 51cm a | 112cm a |
| 100 ppm CCC | 25cm bc | 44cm a | 102cm a |
| 250 ppm CCC | 26cm ab | 45cm a | 101cm a |
| 500 ppm CCC | 20cm bc | 36cm b | 88cm b |

*Analysis of variance table*

| | | *1 week* | | *2 weeks* | | *4 weeks* | |
|---|---|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 53.00 | 7.02** | 147.00 | 8.67** | 418.00 | 6.74* |
| Treatments | 3 | 64.00 | 8.48** | 152.00 | 8.94** | 386.00 | 6.22* |
| Error | 9 | 7.55 | | 17.00 | | 62.00 | |

* Significant at 5% level.

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 8

The effects of CCC on the height of Cucumis sativus (Marketer) grown under glass during the summer of 1965.

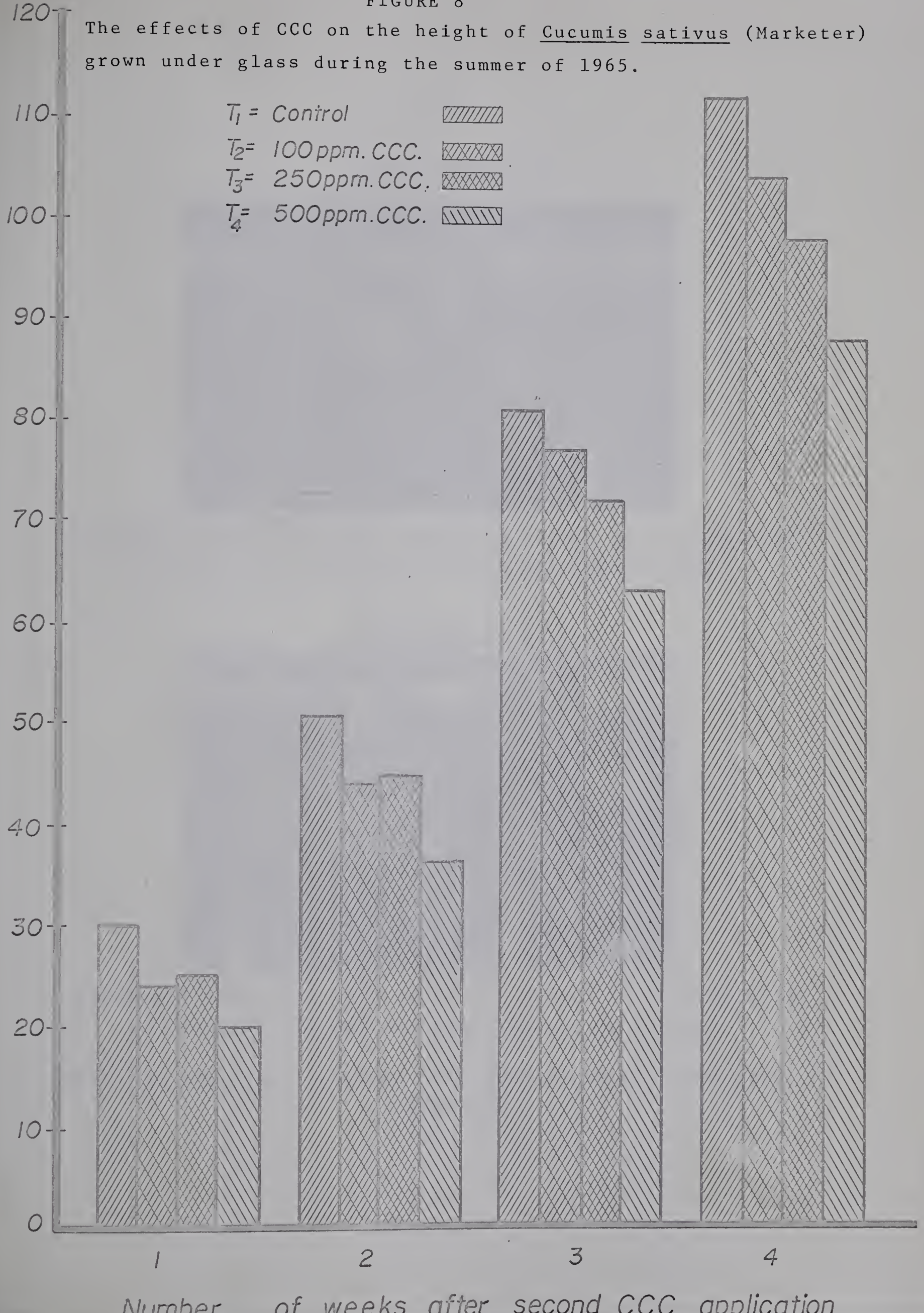


Illustration 10.


Cucumber plants ten days after the second CCC application. $T_1$ control, $T_4$ received 500 ppm CCC application.†

Illustration 11.


Cucumber plants ten days after the second CCC application. $T_3$ received 250 ppm CCC application, $T_1$ control.†

†Note the reduction of plant size due to the CCC treatment. (Marketer).

TABLE XIV

*Average heights of Cucumis sativus L. (Hybrid Burpee) one, three and four weeks after the second CCC application.*

| *Treatments* | *Heights in cms.* | | |
|---|---|---|---|
| | *1 week* | *3 weeks* | *4 weeks* |
| | *After the second CCC application* | | |
| 0 ppm CCC | 43 a† | 119 a | 161 a |
| 100 ppm CCC | 33 b | 102 b | 142 b |
| 250 ppm CCC | 25 c | 86 bc | 131 b |
| 500 ppm CCC | 19 c | 78 c | 115 c |

*Analysis of variance table*

| | | *1 week* | | *3 weeks* | | *4 weeks* | |
|---|---|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 70.60 | 2.86 | 425.00 | 3.93* | 104.00 | 4.38* |
| Treatments | 3 | 442.60 | 17.99** | 1,272.00 | 11.77** | 1,524.00 | 16.56** |
| Error | 9 | 27.60 | | 108.00 | | 92.00 | |

* Significant at 5% level.

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE XV

*Effects of CCC on the number of nodes preceeding the development of the first female flower of Cucumis sativus L. (Hybrid Burpee)*

| *Treatments* | *No. of nodes to first female flower* |
|---|---|
| 0 ppm CCC | 10.2 a |
| 100 ppm CCC | 9.55 a |
| 250 ppm CCC | 8.83 a |
| 500 ppm CCC | 9.30 a |

*Analysis of variance table*

| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 3 | .33 | |
| Treatments | 3 | 1.33 | 1.00 |
| Error | 9 | 1.33 | |

The differences between the location of the first female flower were not significantly different.

TABLE XVI

*Effects of CCC on the number of nodes preceeding the development of the first female flower of Cucumis sativus L. (Marketer)*

| *Treatments* | *No. of nodes to first female flower* |
|---|---|
| 0 ppm CCC | 9.47 a† |
| 100 ppm CCC | 7.85 b |
| 250 ppm CCC | 8.62 a |
| 500 ppm CCC | 8.57 a |

*Analysis of variance table*

| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 3 | .33 | |
| Treatments | 3 | 1.66 | 2.15 |
| Error | 9 | .77 | |

† Numbers in each colun which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

Burpee, the decrease was statistically significant only when 500 ppm CCC was applied. The other CCC treated plants had fewer male flowers also, but these differences were not significant.

The observations pertaining to male and female flowers are presented in Table XVII for Marketer, and in Table XVIII for Hybrid Burpee.

iv. Effects of CCC on the total yield of cucumber plants: All applications of CCC reduced the total yields of Marketer cucumbers. However, this decrease was significant only in plants which received the 500 ppm CCC application and they yielded about 50% less than the control. The Hybrid Burpee variety responded in a similar manner. The plants treated with 100 ppm CCC showed a slightly increased yield, but the difference was not significant. Yields of plants which received the 500 ppm CCC treatments were also reduced about 50% compared to control. The results are summarized in Table XIX for Marketer and Table XX for Hybrid Burpee.

TABLE XVII

*Effects of CCC on the sex expression of* *Cucumis sativus* *L. (Marketer)*

| *Treatments* | *No. of female flowers June 8 to July 27* | *No. of male flowers June 8 to July 27* |
|---|---|---|
| 0 ppm CCC | 51 a† | 215 a |
| 100 ppm CCC | 41 b | 118 b |
| 250 ppm CCC | 44 ab | 112 b |
| 500 ppm CCC | 40 b | 107 b |

*Analysis of variance table*

| | | *Female flowers* | | *Male flowers* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 54.00 | | 1,057.00 | |
| Treatments | 3 | 96.00 | 3.09 | 10,659.00 | 20.98** |
| Error | 9 | 31.00 | | 508.00 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE XVIII

*Effects of CCC on the sex expression of Cucumis sativus L. (Hybrid Burpee).*

| *Treatments* | *No. of female flowers June 8 to July 27* | *No. of male flowers June 8 to July 27* |
|---|---|---|
| 0 ppm CCC | 34.75 ab† | 317.00 a |
| 100 ppm CCC | 43.75 a | 287.00 ab |
| 250 ppm CCC | 33.50 ab | 297.00 ab |
| 500 ppm CCC | 23.75 b | 218.00 b |

*Analysis of variance table*

| | | *Female flowers* | | *Male flowers* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 33.33 | | 1,998.00 | |
| Treatments | 3 | 267.66 | 5.50* | 7,080.00 | 2.43 |
| Error | 9 | 48.66 | | 2,909.00 | |

* Significant at 5% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE XIX

*Effects of CCC on the yield of Cucumis sativus L. (Marketer).*

| *Treatments* | *Av. yields in kg.* | *Av. yields in No.* |
|---|---|---|
| 0 ppm CCC | 4.87 a† | 20.50 a |
| 100 ppm CCC | 3.40 ab | 18.75 ab |
| 250 ppm CCC | 4.13 a | 19.50 ab |
| 500 ppm CCC | 2.46 b | 13.75 b |

*Analysis of variance table*

| | | *Av. yields in kg.* | | *Av. yields in No.* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 1.00 | | 10.70 | |
| Treatments | 3 | 4.00 | 4.54* | 36.00 | 2.32 |
| Error | 9 | .88 | | 15.50 | |

* Significant at 5% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE XX

*Effects of CCC on the yield of Cucumis sativus L. (Hybrid Burpee)*

| *Treatments* | *Av. yields in kg.* | *Av. yields in No.* |
|---|---|---|
| 0 ppm CCC | 5.27 a† | 17.00 a |
| 100 ppm CCC | 6.16 ab | 20.25 a |
| 250 ppm CCC | 4.91 ab | 18.25 a |
| 500 ppm CCC | 2.85 c | 13.20 b |

*Analysis of variance table*

| | | *Av. yields in kg.* | | *Av. yields in No.* | |
|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* |
| Replications | 3 | 1.66 | | 3.66 | |
| Treatments | 3 | 8.00 | 8.00** | 34.66 | 2.73 |
| Error | 9 | 1.00 | | 12.66 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

2. Experiment No. 2.

a) Materials and Methods: The experiment was repeated with the same varieties (Marketer and Hybrid Burpee) and the same methods as described in Experiment No. 1. The bench was steam sterilized before the second experiment. The concentrations of CCC used in the second experiment were lower than in the first, because CCC in the 500 ppm in the first experiment retarded the plants excessively.

| Treatment | Concentrations. |
|---|---|
| 1 | 0 ppm CCC |
| 2 | 100 ppm CCC |
| 3 | 200 ppm CCC |
| 4 | 300 ppm CCC |

The following observations were made:

a) The heights of the plants were measured at weekly intervals for four weeks.

b) Male and female flowers were counted twice per week.

b) Results: In this experiment the plants did not show the same responses to CCC treatments as described in the first greenhouse experiments.

The height measurements were rather inconsistent, especially plants from the Hybrid Burpee variety. The flower counts also failed to indicate any effect due to CCC treatments.

For these reasons, the experiment was abandoned and a new experiment was started.

3. Experiment No. 3.

a) Materials and Methods: This experiment was repeated in the same manner as described in experiment No. 1., with the following exceptions. Only the Marketer variety was used; the Hybrid Burpee was dropped because the seedlings did not show a uniform response to CCC treatments.

The CCC concentrations were as follows:

| Treatment | Concentrations. |
|---|---|
| 1 | 0 ppm CCC |
| 2 | 100 ppm CCC |
| 3 | 200 ppm CCC |
| 4 | 300 ppm CCC |

Each treatment was replicated eight times to increase the plant population in the experiment.

This experiment was conducted under short day conditions. Plants received supplementary light for 9 hours per day (8:00 a.m. to 5:00 p.m.). From 5:00 p.m. to 8:00 a.m. the plants were covered with balck cloth because the neon lights outside the greenhouse illuminated the greenhouse during the night. The day temperature in the greenhouse was 85°F and the night temperature was 70-75°F.

The following observations were made:

a) The heights of the plants were measured four times at weekly intervals, starting one week after the second CCC application.

b) The node number preceeding the development of the first

female flower was noted.

c) Male and female flowers were counted twice a week from December 28/65 to January 17/66.

b) Results:

i. Effects of CCC on the heights of cucumber plants: The heights of the plants in each treatment were quite uniform. Treated plants were reduced in height significantly in all cases compared to control. These differences were evident throughout the growing season. The results are presented in Table XXI and Figure 9.

ii. Effects of CCC on the number of nodes preceeding the development of the first female flower: CCC treatments at 200 ppm concentration significantly reduced the node number to the first female flower, compared to control. The control produced the first female flower at 10.96 node number, while plants receiving the 200 ppm CCC treatments reduced the node number to 9.55. The other two treatments failed to flower at a lower internode number compared to control. The results are presented in Table XXII.

iii. Effects of CCC on the sex expression of cucumbers: CCC treatments failed to significantly increase the formation of female flowers in these experiments. The male flowers, however, were decreased due to CCC treatments. The results are very similar to those obtained during the summer from the first greenhouse experiments, and are summarized in Table XXIII.

TABLE XXI

*Average heights of Cucumis sativus L. (Marketer) one, three and four weeks after the second CCC application*

| *Treatments* | *Heights in cms.* | | |
|---|---|---|---|
| | *1 week* | *3 weeks* | *4 weeks* |
| | *After the second CCC application* | | |
| 0 ppm CCC | 21 a† | 66 a | 92 a |
| 100 ppm CCC | 16 b | 41 b | 65 b |
| 200 ppm CCC | 16 b | 42 b | 57 b |
| 300 ppm CCC | 14 c | 39 b | 54 b |

*Analysis of variance table*

| | | *1 week* | | *3 weeks* | | *4 weeks* | |
|---|---|---|---|---|---|---|---|
| *Source* | *d.f.* | *MS* | *F* | *MS* | *F* | *MS* | *F* |
| Replications | 7 | 5.85 | 4.57* | 159.00 | 4.54* | 69.00 | |
| Treatments | 3 | 80.66 | 63.01** | 1,297.00 | 37.05** | 2,362.00 | 18.74** |
| Error | 21 | 1.28 | | 35.00 | | 126.00 | |

* Significant at 5% level.

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

FIGURE 9.

The effects of CCC on the height of Cucumis sativus (Marketer) grown under glass during the winter of 1965/66.

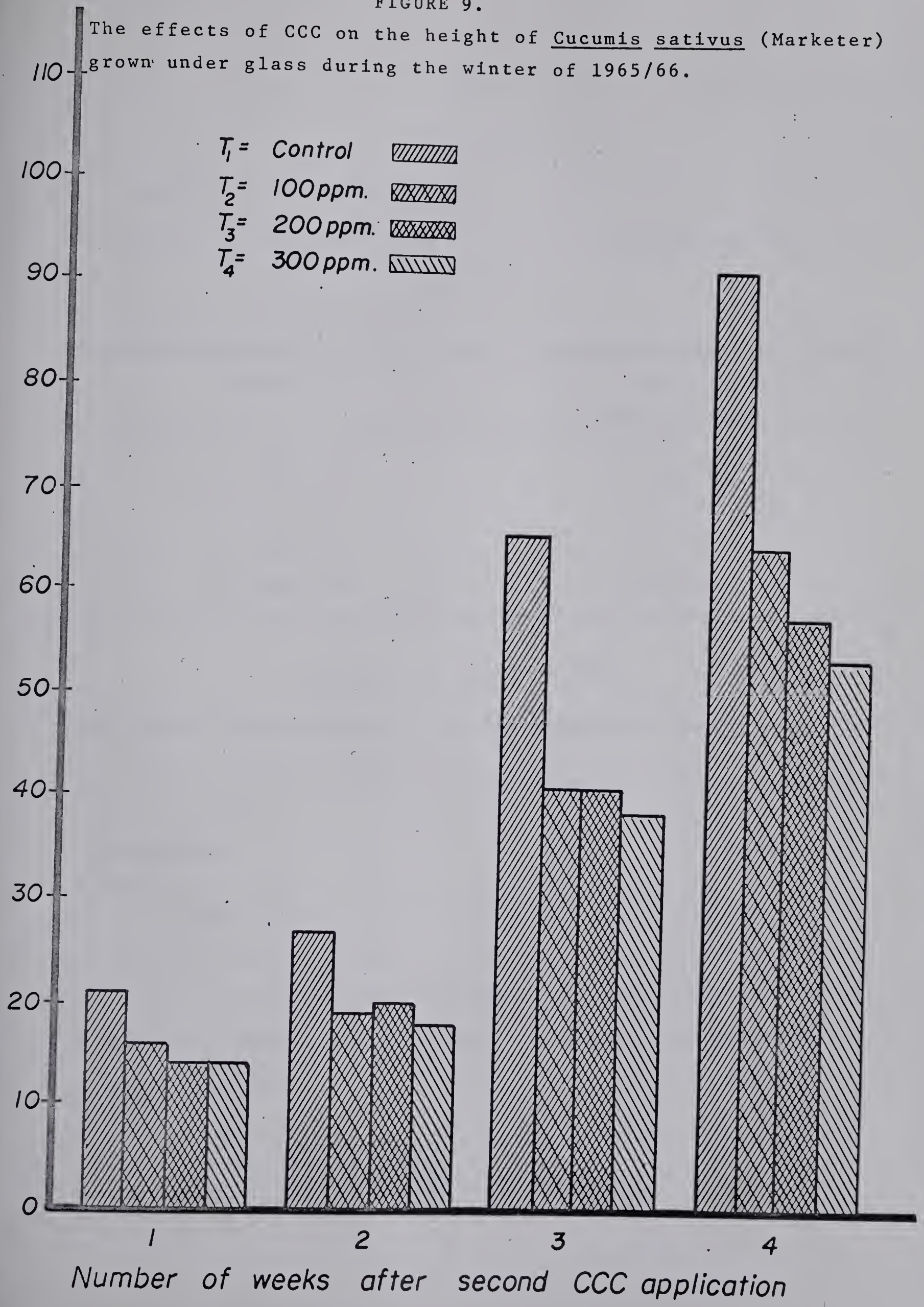


TABLE XXII

*Effects of CCC on the number of nodes preceeding the development of the first female flower of Cucumis sativus L. (Marketer)*

| *Treatments* | *No. of nodes to first female flower* |
|---|---|
| 0 ppm CCC | 10.96 a† |
| 100 ppm CCC | 10.62 a |
| 200 ppm CCC | 9.55 b |
| 300 ppm CCC | 10.25 ab |

*Analysis of variance table*

| *Source* | *d.f.* | *MS* | *F* |
|---|---|---|---|
| Replications | 7 | 1.00 | |
| Treatments | 3 | 3.00 | 3.33 |
| Error | 21 | .90 | |

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

TABLE XXIII

*Effects of CCC on the sex expression of* *Cucumis sativus* *L. (Marketer)*

| *Treatments* | *No. of female flowers Jan 3 - 17* | *No. of male flowers Jan 3 - 17* |
|---|---|---|
| 0 ppm CCC | 4.1 a† | 46.00 a |
| 100 ppm CCC | 5.1 a | 37.00 b |
| 200 ppm CCC | 5.3 a | 35.00 b |
| 300 ppm CCC | 4.5 a | 29.00 c |

*Analysis of variance table*

| *Source* | *d.f.* | *Female flowers* *MS* | *F* | *Male flowers* *MS* | *F* |
|---|---|---|---|---|---|
| Replications | 7 | 3.00 | | 34.14 | |
| Treatments | 3 | 1.00 | - | 406.66 | 35.30** |
| Error | 21 | 1.14 | | 11.52 | |

** Significant at 1% level.

† Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test (34).

C. DISCUSSION AND CONCLUSIONS.

The purpose of this experiment was to study the effects of CCC on the sex expression of cucumber plants.

Flower sex expression in cucumbers is subject to genetic and non-genetic (environmental and chemical) control. Alteration of the length or duration of any of the flower sex stages, but not necessarily the order of appearance, has been modified by environment. The staminate stage has been extended by long photoperiods. The appearance of the pistillate stage has been accelerated by low temperatures, short days and low light intensities. Several chemicals, when applied to the young cucumber seedlings also modify sex expression.

Wittwer (37) reported an increase in the formation of female flowers of Marketer cucumbers treated with a soil drench of $10^{-3}$M CCC solution. Work done by Cathey and Stuart (5,37) with cucumbers does not support the results presented by Wittwer. They reported a reduction in plant size with no increase in the number of female flowers. In the present experiment CCC failed to induce the formation of more female flowers. The results were similar with both field and greenhouse grown plants. On the other hand, CCC treatments in the present study inhibited male flower formation, which is in agreement with results reported by Wittwer (37).

As the monoecious cucumber plant develops, the flower sex gradually changes from staminate to pistillate. The first nodes formed are staminate (staminate stage). The first nodes are

followed by a series of nodes which are truly monoecious (a mixed stage, bearing both staminate and pistillate flowers), and finally successive nodes appear which produce exclusively pistillate flowers (pistillate stage). The number of nodes preceeding the appearance of the first pistillate flower has been used as a criterion of flower sex expression. The fewer the nodes preceeding the first pistillate flower, the shorter the staminate stage and the earlier the mixed stage.

It has also been reported that CCC and related compounds (5,26) reduced the number of nodes preceeding the development of the first pistillate flowers. In these investigations it was found that 100 ppm CCC in the first, and 200 ppm in the third experiment reduced the node number to the first pistillate flower significantly in the Marketer variety, while the remainder of the treatments on Marketer and all treatments applied to Hybrid Burpee failed to decrease the node number to the first pistillate flower.

CCC reduced the yields of fruit when applied at the 500 ppm concentration. The yield reduction in both varieties was about 50% compared to the control. It is suggested that this reduction in yield was mainly due to the severe retardation in the growth of the vines. The 100 and 250 ppm CCC concentrations did not affect the yield significantly and the growth of the vines was reduced 20-40%.

In the second and third greenhouse experiments the CCC concentrations were reduced to 100, 200 and 300 ppm because

the 500 ppm concentration proved to be too strong and caused only severe retardation.

CCC treatments significantly reduced the plant size in all cases compared to the control. The reduction in height varied from 20-50%. The retarding effect of CCC on the height of cucumber plants appeared to be greater in the winter than in the summer.

Reduction in height of the plants has been reported by other authors working with CCC on a number of species of plants. They also noted that the effectiveness of the chemical was greater in the winter than in the summer (4,5,28,50).

Wittwer (49) suggested that chemicals are most effective on the sex expression of the cucumber plant when the chemical stimuli were continuously available to the roots, through a solution culture technique. It is possible that Wittwer supplied the soil medium of the cucumber plants with a continuous supply of CCC, and this might explain his results regarding the increase of pistillate flowers.

To apply CCC to the soil medium continuously in the same concentration is extremely difficult from a practical point of view. This might be the reason why the results of this experiment were not the same as anticipated.

From the practical point of view, it would appear that CCC does not have any beneficial effect on the sex expression of cucumbers. However, there may still be an advantage in using CCC treatments commercially at lower concentrations. The 100

ppm and 200 ppm CCC applications reduced the plant size by 20-40% while yields were not significantly affected.

Provided that the yield of treated plants could be maintained at the control level, smaller plants could be field set at a closer spacing. The increase in the number of plants per acre should result in an increase in yields per acre.

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